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(21) International Application Number: PCT/US98/18506 (22) International Filing Date: 21 September 1998 (21.09.98) (30) Priority Data: 60/060,475 30 September 1997 (30.09.97) US (71) Applicant (for all designated States except US): PHARMACIA & UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): MILLS, Cynthia, J. [US/US]; 8866 North 14th Street, Kalamazoo, MI 49004 (US). JONES, David, A. [US/US]; 1780 E. Logan Avenue, Salt Lake City, UT 84108 (US). BIENKOWSKI, Michael, J. [US/US]; 3431 Hollow Wood, Portage, MI 49024 (US). (74) Agent: KERBER, Lori, L.; Pharmacia & Upjohn Company, Intellectual Property Legal Services, 301 Henrietta Street, Kalamazoo, MI 49001 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: TNF-RELATED DEATH LIGAND (57) Abstract The invention relates to a novel tumor necrosis factor (TNF) homolog designated herein as TNF-related death ligand (TRDL). Isolated nucleic acid molecules are provided which encode TRDL. TRDL polypeptides are also provided, as are methods for identifying agonists and antagonists of TRDL activity.		

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TNF-Related Death Ligand

FIELD OF THE INVENTION

5 The invention relates to a novel tumor necrosis factor (TNF) homolog designated herein as TNF-related death ligand (TRDL).

BACKGROUND OF THE INVENTION

10 Tumor necrosis factor (TNF), named for its ability to shrink tumors, is made by cells of the immune system and is a member of an emerging family of cytokines with important roles in immune regulation, inflammation and cancer. The family includes seven members, in addition to TNF, which share limited sequence homology that is confined to the C-terminal portion of the molecules. With exception of TNF- β , each of these of ligands are type II membrane associated
15 proteins that require cell surface presentation to elicit effects from corresponding target cells (Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C-P., Nicholl, J.K., Sutherland, G.R., Davis Smith, T., Rauch, C., Smith, C.A., and Goodwin, R.G. (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity*. **3**, 673-682). This cytokine family interacts with a
20 growing list of target transmembrane receptors with complicated signaling strategies and often contradictory biological effects (Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C-P., Nicholl, J.K., Sutherland, G.R., Davis Smith, T., Rauch, C., Smith, C.A., and Goodwin, R.G. (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity*. **3**, 673-682).

25 The interaction of TNF- α with the TNFR-1 receptor typifies the biological diversity of the TNF ligand receptor family. Ligation of TNFR-1 can activate NF- κ B and elicit an inflammatory response in a variety of cell types (Beg, A.A. and Baltimore, D. (1996) An essential role for NF- κ B in preventing TNF- α -induced cell death. *Science*. **274**, 782-784. Wang, C-Y., Mayo, M.W., and Baldwin, A.S. Jr. (1996) TNF-and cancer therapy-induced apoptosis: potentiation by inhibition of NF- κ B.
30 *Science*. **274**, 784-787. VanAntwerp, D.J., Martin, S.J., Kafri, T., Green, D.R., and Verma, I.M. (1996) Suppression of TNF- α -induced apoptosis by NF- κ B. *Science*. **274**, 787-789).

 Alternatively, TNFR-1 activation can also induce apoptosis (Pan, G.,
35 O'Rourke, K., Chinnaiyan, A.M., Gentz, R., Ebner, R., Ni, J., and Dixit, V. (1997) The receptor for the cytotoxic ligand TRAIL. *Science*. **276**, 111-113). The mechanisms

- that regulate these two pathways and the final cellular outcome remain unclear, but offer interesting prospects for therapeutic intervention. The importance of this life death balance in human disease can be seen in the observation that TNF fails to efficiently kill many types of cancer cells (Wang, C-Y., Mayo, M.W., and Baldwin, A.S. Jr. (1996) TNF-and cancer therapy-induced apoptosis: potentiation by inhibition of NF- κ B. *Science*. **274**, 784-787. Baichwal, V.R., and Baeuerle, P.A. (1997) Apoptosis: Activate NF- κ B or die? *Current Biology*. **7**, R94-R96). Recent evidence suggests that TNF undermines its own killing powers by activating NF- κ B, a key molecule that can block the apoptosis pathway (Beg, A.A. and Baltimore, D. (1996) An essential role for NF- κ B in preventing TNF- α -induced cell death. *Science*. **274**, 782-784. Wang, C-Y., Mayo, M.W., and Baldwin, A.S. Jr. (1996) TNF-and cancer therapy-induced apoptosis: potentiation by inhibition of NF- κ B. *Science*. **274**, 784-787. VanAntwerp, D.J., Martin, S.J., Kafri, T., Green, D.R., and Verma, I.M. (1996) Suppression of TNF- α -induced apoptosis by NF- κ B. *Science*. **274**, 787-789. Liu, Z-G., Hsu, H., Goeddel, D., and Karin, M. (1996) Dissection of the TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF- κ B activation prevents cell death. *Cell*. **87**, 565-576. Wu, M., Lee, H., Bellas, R.E., Schauer, S.L., Arsura, M., Katz, D., FitzGerald, M.J., Rothstein, T.L., Sherr, D.H., and Sonenshein, G.E. (1996) Inhibition of NF- κ B/Rel induces apoptosis of murine B cells. *EMBO J*. **15**, 4682-4690). This blockade may render tumor cells resistant to immune surveillance and confound chemotherapeutic approaches that rely on tumor cell apoptosis. Disruption of this protective mechanism may, therefore, sensitize cells to TNF mediated killing (Beg, A.A. and Baltimore, D. (1996) An essential role for NF- κ B in preventing TNF- α -induced cell death. *Science*. **274**, 782-784. Wang, C-Y., Mayo, M.W., and Baldwin, A.S. Jr. (1996) TNF-and cancer therapy-induced apoptosis: potentiation by inhibition of NF- κ B. *Science*. **274**, 784-787. VanAntwerp, D.J., Martin, S.J., Kafri, T., Green, D.R., and Verma, I.M. (1996) Suppression of TNF- α -induced apoptosis by NF- κ B. *Science*. **274**, 787-789).

Recent advances in the understanding of TNF signaling have elucidated discrete molecular targets for potential blockade of NF- κ B activation and apoptosis in cells responding to TNF (Beg, A.A. and Baltimore, D. (1996) An essential role for NF- κ B in preventing TNF- α -induced cell death. *Science*. **274**, 782-784. Wang, C-Y., Mayo, M.W., and Baldwin, A.S. Jr. (1996) TNF-and cancer therapy-induced apoptosis: potentiation by inhibition of NF- κ B. *Science*. **274**, 784-787. VanAntwerp, D.J., Martin, S.J., Kafri, T., Green, D.R., and Verma, I.M. (1996) Suppression of

- TNF- α -induced apoptosis by NF- κ B. *Science*. **274**, 787-789. Wu, M., Lee, H., Bellas, R.E., Schauer, S.L., Arsura, M., Katz, D., FitzGerald, M.J., Rothstein, T.L., Sherr, D.H., and Sonenshein, G.E. (1996) Inhibition of NF- κ B/Rel induces apoptosis of murine B cells. *EMBO J.* **15**, 4682-4690). These responses are facilitated by the
- 5 recruitment of signaling proteins to activated TNF receptors. Some of these signaling proteins, including TRADD (TNFR1-associated death domain protein), TRAF2 (TNFR-associated protein-2) and RIP (receptor interacting protein kinase) appear to initiate activation of NF- κ B. Chinnaiyan, A.M., O'Rourke, K., Yu, G-L., Lyons, R.H., Garg, M., Duan, D.R., Xing, L., Gentz, R., Ni, J., and Dixit, V.M. (1996)
- 10 Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. *Science*. **274**, 990-992. Recruitment of other proteins, including FADD (Fas-associated death domain protein) and FLICK (FADD-like interleukin converting enzyme) induces apoptosis (Boldin, M.P., Goncharov, T.M., Goltsev, Y.V., and Wallach, D. (1996) Involvement of MACH, a novel MORT1/FADD-interacting
- 15 protease, in Fas/APO-1-and TNF receptor-induced cell death. *Cell*. **85**, 803-815. Muzio, M., Chinnaiyan, A.M., Kischkel, F.C., O'Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J.D., Zhang, M., Gentz, R., Mann, M., Krammer, P.H., Peter, M.E., and Dixit, V. (1996) FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex.
- 20 *Cell*. **85**, 817-827).

This emerging biology has created considerable interest among researchers for potential therapeutic intervention, particularly in cancer, inflammatory diseases, and neurodegenerative disorders. Thus, it will be clear to the skilled artisan that there is a continuing need for novel members of the TNF family of inflammatory

25 cytokines involved in apoptosis and NF- κ B activation.

INFORMATION DISCLOSURE

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- 35 Wu *et al.*, *EMBO J* **15**, 4682-4690 (1996).

- Chinnaiyan *et al.*, *Science* **274**, 990-992 (1996).
Boldin *et al.*, *Cell* **85**, 803-815 (1996).
Muzio *et al.*, *Cell* **85**, 817-827 (1996).
Altschul *et al.*, *J. Mol. Bio.* **215**, 403-410 (1990).
5 Idziorek *et al.*, *J. Immuno. Meth.* **185**:249-258 (1995).
X.M. Wang, *et al.*, *Human Immunol* **37**: 264 (1993).

SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising a
10 polynucleotide encoding a novel TNF homolog designated herein as TRDL, or a
fragment thereof. TRDL polypeptides of the invention include two alternative splice
variants of TRDL, designated herein as TRDL-11 and TRDL-14, which have the
amino acid sequences shown in Figures 3 and 4 (SEQ ID NO:2 and SEQ ID NO:4,
respectively). Preferred fragments of TRDL include the extracellular portion of
15 TRDL-11 and of TRDL-14.

In a preferred embodiment, the nucleic acid molecules comprise a
polynucleotide having the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3. In
another aspect, the invention provides an isolated nucleic acid molecule comprising a
polynucleotide which hybridizes under stringent conditions to a polynucleotide
20 encoding TRDL-11 or TRDL-14, or fragments thereof.

The present invention also provides vectors comprising the isolated nucleic
acid molecules of the invention, host cells into which such vectors have been
introduced, and recombinant methods of obtaining a TRDL polypeptide comprising
culturing the above-described host cell and isolating the TRDL polypeptide.

25 In another aspect, the invention provides isolated TRDL polypeptides, as well
as fragments thereof. In a preferred embodiment, the TRDL polypeptides have the
amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Isolated antibodies, both
polyclonal and monoclonal, that bind specifically to TRDL polypeptides are also
provided.

30 The invention also provides a method for the identification of cells having a
high affinity receptor for TRDL, the method comprising:

- (a) labeling an isolated TRDL polypeptide ;
- (b) contacting the labeled TRDL polypeptide obtained in step (a)
with cells of a mammalian cell line;
- 35 (c) washing the cells obtained in step (b) to remove unbound
TRDL; and

- (d) determining the presence of the labeled TRDL polypeptide in the washed cells obtained in step (c);

whereby the presence of labeled TRDL polypeptide in the washed cells obtained in step (c) indicates the presence of a high affinity receptor for TRDL on the cells.

5 In another embodiment, the invention relates to a method for the identification of an agent which inhibits the binding of TRDL to its receptor, the method comprising:

- (a) labeling an isolated TRDL polypeptide;
- (b) contacting the labeled TRDL polypeptide obtained in step (a)
10 with cells of a mammalian cell line
- (i) in the presence of a test agent; and
- (ii) in the absence of a test agent;
- (c) washing the cells
- (i) obtained in step (b)(i); and
- 15 (ii) obtained in step (b)(ii) to remove unbound TRDL;
- (d) determining the amount of labeled TRDL polypeptide in
- (i) the washed cells obtained in step (c)(i); and
- (ii) the washed cells obtained in step (c)(ii); and
- (e) comparing the amount of labeled TRDL polypeptide determined
20 in step (d)(i) to that determined in (d)(ii);

whereby a lower amount of labeled TRDL polypeptide in sample (d)(i) than in sample (d)(ii) indicates that said agent inhibited the binding of TRDL to its receptor.

In another embodiment, the invention relates to a method for the identification of an agent which enhances the binding of TRDL to its receptor, the
25 method comprising:

- (a) labeling an isolated TRDL polypeptide;
- (b) contacting the labeled TRDL polypeptide obtained in step (a)
with cells of a mammalian cell line
- (i) in the presence of a test agent; and
- 30 (ii) in the absence of a test agent;
- (c) washing the cells
- (i) obtained in step (b)(i); and
- (ii) obtained in step (b)(ii) to remove unbound TRDL;
- (d) determining the amount of labeled TRDL polypeptide in
- 35 (i) the washed cells obtained in step (c)(i); and
- (ii) the washed cells obtained in step (c)(ii); and

- (e) comparing the amount of labeled TRDL polypeptide determined in step (d)(i) to that determined in (d)(ii);

whereby a higher amount of labeled TRDL polypeptide in sample (d)(i) than in sample (d)(ii) indicates that said agent has enhanced the binding of TRDL to its

5 receptor.

In another embodiment, the invention provides A method for the identification of a cell line that undergoes apoptosis upon interaction with TRDL, the method comprising:

- 10 (a) dividing the cells of a culture of a mammalian cell line into a test culture and a control culture;
- (b) contacting a TRDL polypeptide with the test culture of step (a);
- (c) determining the quantity of cells of
- (i) the test culture obtained in step (b); and
- (ii) the control culture of step (a);
- 15 that have undergone apoptosis; and
- (d) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (c)(i) with the quantity of cells determined to have undergone apoptosis in the control culture of step (c)(ii);

20 whereby a determination that the quantity of cells having undergone apoptosis in said test culture is higher than in said control culture indicates that said mammalian cell line undergoes apoptosis upon interaction with TRDL.

In yet another embodiment, the invention provides a method for the identification of an agent capable of inhibiting TRDL-mediated induction of apoptosis, said method comprising

- 25 (a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the presence of a test agent, and said control culture
- 30 comprises mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent; and
- (b) comparing the quantity of cells determined to have undergone
- 35 apoptosis in the test culture of step (a) with the quantity of cells

determined to have undergone apoptosis in the control culture of step
(a);

whereby a determination that the quantity of cells having undergone apoptosis in
said test culture is lower than in said control culture indicates that said test agent
5 inhibits TRDL-mediated induction of apoptosis.

In yet another embodiment, the invention provides a method for the
identification of an agent capable of enhancing TRDL-mediated induction of
apoptosis, said method comprising

- 10 (a) determining the quantity of cells that have undergone apoptosis in
a test culture and a control culture, wherein said test culture
comprises mammalian cells capable of undergoing apoptosis upon
interaction with TRDL which have been contacted with a TRDL
polypeptide in the presence of a test agent, and said control culture
15 comprises mammalian cells capable of undergoing apoptosis upon
interaction with TRDL which have been contacted with a TRDL
polypeptide in the absence of a test agent; and
- (b) comparing the quantity of cells determined to have undergone
apoptosis in the test culture of step (a) with the quantity of cells
determined to have undergone apoptosis in the control culture of step
20 (a);

whereby a determination that the quantity of cells having undergone apoptosis in
said test culture is higher than in said control culture indicates that said test agent
enhances TRDL-mediated induction of apoptosis.

In yet another embodiment, the invention provides a method for the
25 identification of an agent capable of inhibiting TRDL-mediated prevention of
apoptosis, said method comprising:

- 30 (a) determining the quantity of cells that have undergone apoptosis in
a test culture and a control culture, wherein said test culture
comprises mammalian cells that are prevented from undergoing
apoptosis upon interaction with TRDL which have been contacted with
a TRDL polypeptide in the presence of a test agent, and said control
culture comprises mammalian cells that are prevented from
undergoing apoptosis upon interaction with TRDL which have been
contacted with a TRDL polypeptide in the absence of a test agent;
35 and
- (b) comparing the quantity of cells determined to have undergone

apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is higher than in said control culture indicates that said test agent inhibits TRDL-mediated prevention of apoptosis.

In yet another embodiment, the invention provides a method for the identification of an agent capable of enhancing TRDL-mediated prevention of apoptosis, said method comprising

- 10 (a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the presence of a test agent, and said control
15 culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent; and
- 20 (b) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is lower than in said control culture indicates that said test agent
25 enhances TRDL-mediated prevention of apoptosis.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B. Figures 1A and 1B are sequence comparisons showing the
30 results of an Incyte LifeSeq database search for TNF homologs. The database was searched using the basic local alignment tool (BLAST) and the sequence for TNF- α (accession #M10988). Two regions in the coding region of clone 78258 have interesting regions of homology with TNF- α . Figure 1A shows one such region of homology: a region of clone 78258 (SEQ ID NO:5) is compared to a region of TNF- α
35 (SEQ ID NO:6). Figure 1B shows another region of homology: a second region of clone 78258 (SEQ ID NO:7) is compared to a second region of TNF- α (SEQ ID NO:8).

Figure 2. Figure 2 shows cluster assembly and consensus of clones within a 709 bp cDNA sequence. A contiguous 709 bp cDNA sequence was obtained using the LifeSeq Assembly function for the clones in cluster 99092, including clone 78258. Included in the sequence is clone 798247, which lies furthest upstream in the contig.

5

Figure 3. Figure 3 shows the nucleotide sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of TRDL-11.

Figure 4. Figure 4 shows the nucleotide sequence (SEQ ID NO:3) and predicted amino acid sequence (SEQ ID NO:4) of TRDL-14.

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Figure 5. Figure 5 shows a DNA sequence alignment of the nucleotide sequences of clones TRDL-11 (SEQ ID NO:1) and TRDL-14 (SEQ ID NO:3). Clones 11 & 14 are nearly identical with the exception of a 48 base pair stretch present in clone 14 which is missing from clone 11, and 183 base pairs 5' present at the 3' end of clone 11 which are absent in clone 14.

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Figure 6. Figure 6 shows an alignment of the predicted amino acid sequences encoded by clones TRDL-11 (SEQ ID NO:2) and TRDL-14 (SEQ ID NO:4), to the amino acid sequences of TNF family members TNF- α (SEQ ID NO:9), FasL (SEQ ID NO:10), and TRAIL (SEQ ID NO:11). Regions of homology are highlighted in gray.

20

Figure 7. Figure 7 shows the results of electronic northern blots for clones 177393 and 798247. These 2 clones are representative members of the clusters which encompass the sequence for TRDL-11 (SEQ ID NO:1) and TRDL-14 (SEQ ID NO:3). They are expressed mainly in inflammatory tissues and tissues with an abundant peripheral blood supply.

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Figures 8A and 8B. Figure 8A is a northern blot showing the tissue distribution of expression of clone 798247. A multiple tissue Northern analysis was carried out using full-length 798247 as the probe. Two separate blots representing the indicated tissues (1=heart, 2=brain, 3=placenta, 4=lung, 5=liver, 6=skeletal muscle, 7=kidney, 8=pancreas, 9=spleen, 10=thymus, 11=prostate, 12=testis, 13=ovary, 14=small intestine, 15=colon, 16=peripheral blood leukocytes) were analyzed in parallel. The probe hybridized to messages at approximately 1.6 & 1.8 kb. Signals were quantified using a phosphorimager, and are displayed in the plot as relative

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phosphorescence. Figure 8B is a graph showing the relative phosphorescence of the labeled 798247 probe in northern blots from the indicated tissue type.

- Figures 9A and 9B. Figure 9A is a northern blot showing the distribution of TRDL-14 expression in human cancer cell lines. A multiple cancer cell line Northern analysis (1=HL-60, 2=HeLaS3, 3=K-562, 4=MOLT-4, 5=BL Raji, 6=SW480, 7=A549, and 8=G361) was carried out using the coding region of TRDL-14 as the probe. The probe hybridized to messages at approx. 1.7 kb (bars 1 & 2) and 2.5 kb (bars 3 & 4) in HeLa and SW480 cells. A third mRNA species at 4.4 kb was moderately expressed in HeLa, SW480, HL-60, and K-562 cells, and at low levels in MOLT-4, BL Raji, A549, and G351 cells (bars 5-12). Figure 9B is a graph showing the relative phosphorescence of the labeled 798247 probe in northern blots from the indicated cancer cell line.
- Figures 10A and 10B. Figure 10A is a graph showing the distribution of TRDL-14 expression in normal tumor tissue samples. High levels of expression were seen in tumor samples from duodenum & colon. Expression in lung was high in both normal and tumor samples. Figure 10B is a graph showing the distribution of TRDL-14 expression in normal and tumor tissue samples. A high level of expression was seen in the tumor sample from pancreas. Expression was higher in normal than tumor samples from gallbladder, thymus, adrenal, breast, and thyroid.

DETAILED DESCRIPTION

The present invention provides isolated nucleic acid molecules comprising polynucleotides encoding two alternative splice variants of the TRDL polypeptide, designated herein as TRDL-11 and TRDL-14, and having the amino acid sequence shown in Figures 4 and 5 (SEQ ID NO:2 and SEQ ID NO:4, respectively). Unless otherwise indicated, all references to TRDL are to be understood as referring to either TRDL-11 or TRDL-14.

The TRDL polypeptides of the present invention share sequence homology with the family of cytokines which includes TNF- α , FasL, and TRAIL. Sequence conservation with molecules like TNF, TRAIL and FasL is confined primarily to two short stretches within 100 amino acids from the carboxy terminus. The overall percent identity of the C-terminal domain of TRDL to TNF α , FasL and TRAIL is 17%, 17% and 12%, respectively. Although not striking, this level of homology is typical of the TNF cytokine family. By comparison, TRAIL is only 28% homologous

to FasL, 23% homologous to TNF α and 22% homologous to lymphotoxin β . Like these family members, TRDL contains a putative transmembrane segment near the N-terminus that is likely required for proper presentation of this molecule on cell surfaces. Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C-P., Nicholl, J.K., Sutherland, G.R., Davis Smith, T., Rauch, C., Smith, C.A., and Goodwin, R.G. (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity*. 3, 673-682.

Structural domains of the TRDL polypeptides of the present invention have been predicted via the Transmem program (Pharmacia & Upjohn, Kalamazoo, MI). Thus, the structural domains of TRDL-11, whose amino acid sequence is given in SEQ ID NO:2, are predicted to comprise an N-terminal cytoplasmic domain corresponding to amino acid residue 1 to about amino acid residue 23, a transmembrane region corresponding to about amino acid residue 24 to about amino acid residue 52, and a C-terminal extracellular domain corresponding to about amino acid residue 53 to about amino acid residue 234. The structural domains of TRDL-14, whose amino acid sequence is given in SEQ ID NO:4, are predicted to comprise an N-terminal cytoplasmic domain corresponding to amino acid residue 1 to about amino acid residue 23, a transmembrane region corresponding to about amino acid residue 24 to about amino acid residue 52, and a C-terminal extracellular domain corresponding to about amino acid residue 53 to about amino acid residue 247. The invention thus also provides nucleic acid molecules which encode the intracellular, transmembrane, or extracellular region of either TRDL-11 or TRDL-14.

As will be understood by the skilled artisan, the transmembrane region of each TRDL polypeptide described above is identified in accordance with conventional criteria for identifying that type of hydrophobic domain. Of course, the exact boundaries of the transmembrane region may vary slightly from those given above. Thus, the N-terminal boundary of the transmembrane domain of TRDL may begin 5 amino acid residues N-terminal to or C-terminal to the designated boundary, and the C-terminal boundary of the transmembrane domain of TRDL may begin 5 amino acid residues N-terminal to or C-terminal to the designated boundary. Of course, where the actual boundaries of the transmembrane region are shifted as described above, corresponding shifts in the C-terminal boundary of the intracellular domain and the N-terminal boundary of the extracellular domain will also be found.

The nucleotide sequences given in SEQ ID NO:1 and SEQ ID NO:3 correspond to the nucleotide sequences encoding TRDL-11 and TRDL-14,

respectively. The isolation and sequencing of DNA encoding TRDL is described below at Example 1. The DNA of SEQ ID NO:1 (encoding TRDL-11), as compared to the DNA of SEQ ID NO:3 (encoding TRDL-14), contains an in-frame deletion of about 48 base pairs that encodes about 16 amino acids (about residue 111 to about residue 127 of TRDL-14) from the DNA of SEQ ID NO:3 (TRDL-14). The DNA of
5 SEQ ID NO:3 (encoding TRDL-14), as compared to the DNA of SEQ ID NO:1 (encoding TRDL-11), lacks about 183 base pairs near the termination codon from the DNA of SEQ ID NO:1 (TRDL-11) (see Figure 5).

As is described in Example 1, automated sequencing methods were used to
10 obtain the nucleotide sequence of TRDL. The TRDL nucleotide sequences of the present invention were obtained for both DNA strands, and are believed to be 100% accurate. However, as is known in the art, nucleotide sequence obtained by such automated methods may contain some errors. Nucleotide sequences determined by automation are typically at least about 90%, more typically at least about 95% to at
15 least about 99.9% identical to the actual nucleotide sequence of a given nucleic acid molecule. The actual sequence may be more precisely determined using manual sequencing methods, which are well known in the art. As is known in the art, an error in sequence which results in an insertion or deletion of one or more nucleotides may result in a frame shift in translation such that the predicted amino acid
20 sequence will differ from that which would be predicted from the actual nucleotide sequence of the nucleic acid molecule, starting at the point of the mutation.

The TRDL DNA of the present invention includes cDNA, chemically synthesized DNA, DNA isolated by PCR, genomic DNA, and combinations thereof. Genomic TRDL DNA may be obtained by screening a genomic library with the
25 TRDL cDNA described herein (See Example 3, section (e)). RNA transcribed from TRDL DNA is also encompassed by the present invention.

Due to the degeneracy of the genetic code, two DNA sequences may differ and yet encode identical amino acid sequences. The present invention thus provides isolated nucleic acid molecules having a polynucleotide sequence encoding any of the
30 TRDL polypeptides of the invention, wherein said polynucleotide sequence encodes a TRDL polypeptide having the complete amino acid sequence of SEQ ID NO:2 or SEQ ID NO: 4, or fragments thereof.

Also provided herein are purified TRDL polypeptides, both recombinant and non-recombinant. Variants and derivatives of native TRDL proteins that retain any
35 of the biological activities of TRDL are also within the scope of the present invention. One biological activity of TRDL is the ability to induce apoptosis. Assay

procedures for detecting apoptosis of target cells are well known. These include the DNA laddering apoptosis assay and cell lysis assays described by Wiley *et al.* (WO 97/01633).

TRDL variants may be obtained by mutation of native TRDL-encoding
5 nucleotide sequences, for example. A TRDL variant, as referred to herein, is a polypeptide substantially homologous to a native TRDL but which has an amino acid sequence different from that of native TRDL because of one or more deletions, insertions, or substitutions in the amino acid sequence. The variant amino acid or
10 nucleotide sequence is preferably at least about 80% identical, more preferably at least about 90% identical, and most preferably at least about 95% identical, to a native TRDL sequence. The percentage of sequence identity, also termed homology, between a native and a variant TRDL sequence may be determined, for example, by comparing the two sequences using any of the computer programs commonly employed for this purpose, such as the Bestfit program (Wisconsin Sequence
15 Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wisconsin), which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.* 2: 482-489 (1981)).

Alterations of the native amino acid sequence may be accomplished by any of a number of known techniques. For example, mutations may be introduced at
20 particular locations by procedures well known to the skilled artisan, such as oligonucleotide-directed mutagenesis, which is described by Walder *et al.* (*Gene* 42:133 (1986)); Bauer *et al.* (*Gene* 37:73 (1985)); Craik (*BioTechniques*, January 1985, pp. 12-19); Smith *et al.* (*Genetic Engineering: Principles and Methods*, Plenum Press (1981)); and U.S. Patent Nos. 4,518,584 and 4,737,462.

25 TRDL variants within the scope of the invention may comprise conservatively substituted sequences, meaning that one or more amino acid residues of a TRDL polypeptide are replaced by different residues that do not alter the secondary and/or tertiary structure of the TRDL polypeptide. Such substitutions may include the replacement of an amino acid by a residue having similar physicochemical
30 properties, such as substituting one aliphatic residue (Ile, Val, Leu or Ala) for another, or substitution between basic residues Lys and Arg, acidic residues Glu and Asp, amide residues Gln and Asn, hydroxyl residues Ser and Tyr, or aromatic residues Phe and Tyr. Further information regarding making phenotypically silent amino acid exchanges may be found in Bowie *et al.*, *Science* 247:1306-1310 (1990).
35 Other TRDL variants which might retain substantially the biological activities of TRDL are those where amino acid substitutions have been made in areas outside

functional regions of the protein, for example, in a region falling outside of a receptor binding site.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent conditions to a portion
5 of the nucleic acid molecules described above, e.g. to at least about 15 nucleotides, preferably to at least about 20 nucleotides, more preferably to at least about 30 nucleotides, and still more preferably to at least about from 30 to at least about 100 nucleotides, of one of the previously described nucleic acid molecules. Such portions of nucleic acid molecules having the described lengths refer to, e.g., at least about 15
10 contiguous nucleotides of the reference nucleic acid molecule. By stringent hybridization conditions is intended overnight incubation at about 42°C for about 2.5 hours in 6 X SSC/0.1% SDS, followed by washing of the filters in 1.0 X SSC at 65°C, 0.1% SDS.

Fragments of the TRDL-encoding nucleic acid molecules described herein, as
15 well as polynucleotides capable of hybridizing to such nucleic acid molecules (including the fragments which encode the intracellular, transmembrane, or extracellular region of any of TRDL-1, TRDL-11, and TRDL-14) may be used as a probe or as primers in a polymerase chain reaction (PCR). Such probes may be used, e.g., to detect the presence of TRDL nucleic acids in *in vitro* assays, as well as
20 in Southern and northern blots. Cell types expressing TRDL may also be identified by the use of such probes. Such procedures are well known, and the skilled artisan will be able to choose a probe of a length suitable to the particular application. For PCR, 5' and 3' primers corresponding to the termini of a desired TRDL nucleic acid molecule are employed to isolate and amplify that sequence using conventional
25 techniques.

Other useful fragments of the TRDL nucleic acid molecules are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence capable of binding to a target TRDL mRNA (using a sense strand), or TRDL DNA (using an antisense strand) sequence.

30 In another aspect, the invention includes TRDL polypeptides with or without associated native pattern glycosylation. TRDL expressed in yeast or mammalian expression systems (discussed below) may be similar to or significantly different from a native TRDL polypeptide in molecular weight and glycosylation pattern. Expression of TRDL in bacterial expression systems will provide non-glycosylated
35 TRDL.

The polypeptides of the present invention are preferably provided in an

isolated form, and preferably are substantially purified. TRDL polypeptides may be recovered and purified from recombinant cell cultures by well-known methods, including ammonium sulfate or ethanol precipitation, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction
5 chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. In a preferred embodiment, high performance liquid chromatography (HPLC) is employed for purification.

The present invention also relates to vectors comprising the polynucleotide molecules of the invention, as well as host cell transformed with such vectors. Any of
10 the polynucleotide molecules of the invention may be joined to a vector, which generally includes a selectable marker and an origin of replication, for propagation in a host. Because the invention also provides TRDL polypeptides expressed from the polynucleotide molecules described above, vectors for the expression of TRDL are preferred. The vectors include DNA encoding any of the TRDL polypeptides
15 described above or below, operably linked to suitable transcriptional or translational regulatory sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, mRNA ribosomal binding sites, and appropriate sequences which control transcription and translation. Nucleotide sequences are operably
20 linked when the regulatory sequence functionally relates to the DNA encoding TRDL. Thus, a promoter nucleotide sequence is operably linked to a TRDL DNA sequence if the promoter nucleotide sequence directs the transcription of the TRDL sequence.

Selection of suitable vectors to be used for the cloning of polynucleotide
25 molecules encoding TRDL, or for the expression of TRDL polypeptides, will of course depend upon the host cell in which the vector will be transformed, and, where applicable, the host cell from which the TRDL polypeptide is to be expressed. Suitable host cells for expression of TRDL polypeptides include prokaryotes, yeast, and higher eukaryotic cells, each of which is discussed below.

30 The TRDL polypeptides to be expressed in such host cells may also be fusion proteins which include regions from heterologous proteins. Such regions may be included to allow, *e.g.*, secretion, improved stability, or facilitated purification of the polypeptide. For example, a sequence encoding an appropriate signal peptide can be incorporated into expression vectors. A DNA sequence for a signal peptide
35 (secretory leader) may be fused in-frame to the TRDL sequence so that TRDL is translated as a fusion protein comprising the signal peptide. A signal peptide that

is functional in the intended host cell promotes extracellular secretion of the TRDL polypeptide. Preferably, the signal sequence will be cleaved from the TRDL polypeptide upon secretion of TRDL from the cell. Non-limiting examples of signal sequences that can be used in practicing the invention include the yeast α -factor and
5 the honeybee melatin leader in sf9 insect cells.

In a preferred embodiment, the TRDL polypeptide will be a fusion protein which includes a heterologous region used to facilitate purification of the polypeptide. Many of the available peptides used for such a function allow selective binding of the fusion protein to a binding partner. For example, the TRDL
10 polypeptide may be modified to comprise a peptide to form a fusion protein which specifically binds to a binding partner, or peptide tag. Non-limiting examples of such peptide tags include the 6-His tag, thioredoxin tag, hemagglutinin tag, GST tag, and OmpA signal sequence tag. As will be understood by one of skill in the art, the binding partner which recognizes and binds to the peptide may be any molecule or
15 compound including metal ions (e.g., metal affinity columns), antibodies, or fragments thereof, and any protein or peptide which binds the peptide, such as the FLAG tag.

Suitable host cells for expression of TRDL polypeptides include prokaryotes, yeast, and higher eukaryotic cells. Suitable prokaryotic hosts to be used for the
20 expression of TRDL include bacteria of the genera *Escherichia*, *Bacillus*, and *Salmonella*, as well as members of the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. For expression in, e.g., *E. coli*, a TRDL polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in a prokaryotic host. The N-terminal Met may optionally then be
25 cleaved from the expressed TRDL polypeptide.

Expression vectors for use in prokaryotic hosts generally comprise one or more phenotypic selectable marker genes. Such genes generally encode, e.g., a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial
30 sources. Examples include pSPORT vectors, pGEM vectors (Promega), pPROEX vectors (LTI, Bethesda, MD), Bluescript vectors (Stratagene), and pQE vectors (Qiagen).

TRDL may also be expressed in yeast host cells from genera including *Saccharomyces*, *Pichia*, and *Kluveromyces*. Preferred yeast hosts are *S. cerevisiae*
35 and *P. pastoris*. Yeast vectors will often contain an origin of replication sequence from a 2 μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter

region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Vectors replicable in both yeast and *E. coli* (termed shuttle vectors) may also be used. In addition to the above-mentioned features of yeast vectors, a shuttle vector will also include sequences for replication and selection in
5 *E. coli*. Direct secretion of TRDL polypeptides expressed in yeast hosts may be accomplished by the inclusion of nucleotide sequence encoding the yeast α -factor leader sequence at the 5' end of the TRDL-encoding nucleotide sequence.

Insect host cell culture systems may also be used for the expression of TRDL polypeptides. Expression of a TRDL polypeptide in baculovirus is described below in
10 Example 4. Further information regarding the use of baculovirus systems for the expression of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988).

In a preferred embodiment, TRDL polypeptides are expressed in mammalian host cells. Non-limiting examples of suitable mammalian cell lines include the
15 COS-7 line of monkey kidney cells (Gluzman *et al.*, *Cell* 23:175 (1981)) and Chinese hamster ovary (CHO) cells. Expression of TRDL polypeptides in CHO cells is described below in Example 4.

The choice of a suitable expression vector for expression of TRDL polypeptides will of course depend upon the specific mammalian host cell to be used,
20 and is within the skill of the ordinary artisan. Examples of suitable expression vectors include pcDNA3 (Invitrogen) and pSVL (Pharmacia Biotech). Expression vectors for use in mammalian host cells may include transcriptional and translational control sequences derived from viral genomes. Commonly used promoter sequences and enhancer sequences which may be used in the present
25 invention include, but are not limited to, those derived from human cytomegalovirus (CMV), Adenovirus 2, Polyoma virus, and Simian virus 40 (SV40). Methods for the construction of mammalian expression vectors are disclosed, for example, in Okayama and Berg (*Mol. Cell. Biol.* 3:280 (1983)); Cosman *et al.* (*Mol. Immunol.* 23:935 (1986)); Cosman *et al.* (*Nature* 312:768 (1984)); EP-A-0367566; and WO
30 91/18982.

The polypeptides of the present invention may also be used to raise polyclonal and monoclonal antibodies, which are useful in diagnostic assays to for detecting TRDL polypeptide expression. Such antibodies may be prepared by conventional techniques. See, for example, *Antibodies: A Laboratory Manual*,
35 Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988); *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological*

Analyses, Kennet *et al.* (eds.), Plenum Press, New York (1980).

The TRDL nucleic acid molecules of the present invention are also valuable for chromosome identification, as they can hybridize with a specific location on a human chromosome (described in Example 4). There is a current need for
5 identifying particular sites on the chromosome, as few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. The relationship between
10 genes and diseases that have been mapped to the same chromosomal region can then be identified through linkage analysis, wherein the coinheritance of physically adjacent genes is determined. Whether a gene appearing to be related to a particular disease is in fact the cause of the disease can then be determined by comparing the nucleic acid sequence between affected and unaffected individuals.

15 Because TRDL is a new member of the TNF family of inflammatory cytokines, as demonstrated by the sequence homology between TDRL and TNF family members such as Fas ligand and TRAIL, it is likely that TRDL will elicit similar biological responses. These include activation of cellular apoptosis and activation of NF κ B. Certain disease states exhibit a low level of apoptosis as
20 compared to the level of apoptosis occurring in an individual not suffering from the disease. Such diseases include, *e.g.*, cancers with p53 mutations, hormone-dependent tumors, autoimmune disorders and viral infections. Furthermore, as is described below in Example 2, certain tissue types exhibit higher levels of TRDL expression in normal tissue than in tumor tissue. Thus, one use for TRDL relates to
25 a method for treating an individual in need of an increase in TRDL activity comprising administering to said individual TRDL, or an agent capable of decreasing TRDL activity (*i.e.*, a TRDL agonist) to said individual. Administration of TRDL or a TRDL agonist may lead to the desired programmed cell death.

In addition, certain diseases involve an increased level of apoptosis as
30 compared to the level of apoptosis occurring in an individual not suffering from the disease. Administration of TRDL or an agent that decreases TRDL activity (*i.e.*, a TRDL antagonist) may therefore reduce the level of apoptosis in such an individual. Such diseases include, *e.g.*, AIDS, neurodegenerative disorders, myelodysplastic disorders and ischemic injury. Thus, one use for TRDL relates to a method for
35 treating an individual in need of a decrease in TRDL activity comprising administering to said individual an agent capable of decreasing TRDL activity (*i.e.*, a

TRDL antagonist) to said individual. Administration of a TRDL antagonist may lead to the desired programmed cell death.

Thus, in another embodiment, the invention relates to the use of TRDL polypeptides in the identification of TRDL agonists and antagonists. One such use of TRDL polypeptides is described in Example 5, below. In this example, cells bearing high affinity TRDL receptors are identified by combining labeled TRDL with different cell lines. The cell line expressing the highest level of TRDL binding is then used in high-throughput screening assays for TRDL agonists and antagonists as described below. An agent increasing TRDL binding to the cell line is thus a TRDL agonist, while an agent decreasing TRDL binding is a TRDL antagonist. Suitable labels for TRDL include biotin, radioisotopes such as ^{125}I , ^{14}C , ^{35}S , and ^3H , fluorescent labels such as fluorescein and rhodamine, and enzymatic labels.

Thus, the invention provides a method for the identification of cells having a high affinity receptor for TRDL, the method comprising:

- 15 (a) labeling an isolated TRDL polypeptide ;
- (b) contacting the labeled TRDL polypeptide obtained in step (a) with cells of a mammalian cell line;
- (c) washing the cells obtained in step (b) to remove unbound TRDL; and
- 20 (d) determining the presence of the labeled TRDL polypeptide in the washed cells obtained in step (c);

whereby the presence of labeled TRDL polypeptide in the washed cells obtained in step (c) indicates the presence of a high affinity receptor for TRDL on the cells.

In another embodiment, the invention relates to a method for the identification of an agent which inhibits the binding of TRDL to its receptor, the method comprising:

- (a) labeling an isolated TRDL polypeptide;
- (b) contacting the labeled TRDL polypeptide obtained in step (a) with cells of a mammalian cell line
- 30 (i) in the presence of a test agent; and
- (ii) in the absence of a test agent;
- (c) washing the cells
- (i) obtained in step (b)(i); and
- (ii) obtained in step (b)(ii) to remove unbound TRDL;
- 35 (d) determining the amount of labeled TRDL polypeptide in
- (i) the washed cells obtained in step (c)(i); and

- (ii) the washed cells obtained in step (c)(ii); and
- (e) comparing the amount of labeled TRDL polypeptide determined in step (d)(i) to that determined in (d)(ii);

whereby a lower amount of labeled TRDL polypeptide in sample (d)(i) than in
 5 sample (d)(ii) indicates that said agent inhibited the binding of TRDL to its receptor.

In another embodiment, the invention relates to a method for the identification of an agent which enhances the binding of TRDL to its receptor, the method comprising:

- (a) labeling an isolated TRDL polypeptide;
- 10 (b) contacting the labeled TRDL polypeptide obtained in step (a) with cells of a mammalian cell line
 - (i) in the presence of a test agent; and
 - (ii) in the absence of a test agent;
- (c) washing the cells
 - 15 (i) obtained in step (b)(i); and
 - (ii) obtained in step (b)(ii) to remove unbound TRDL;
- (d) determining the amount of labeled TRDL polypeptide in
 - (i) the washed cells obtained in step (c)(i); and
 - (ii) the washed cells obtained in step (c)(ii); and
- 20 (e) comparing the amount of labeled TRDL polypeptide determined in step (d)(i) to that determined in (d)(ii);

whereby a higher amount of labeled TRDL polypeptide in sample (d)(i) than in sample (d)(ii) indicates that said agent has enhanced the binding of TRDL to its receptor.

25 Furthermore, as is described above, it is likely that TRDL will demonstrate activities common to the TNF family of cytokines, such as induction of apoptosis or prevention of apoptosis. Assays to be used for quantitation of cells having undergone apoptosis are well known in the art (*See, e.g., Wiley et al., Immunity* 3:673-682 (1995); Idziorek *et al., J. Immuno. Meth.* 185:249-258 (1995); X.M. Wang,
 30 *et al., Human Immunol* 37: 264 (1993)). Thus, by using an assay such as the DNA laddering apoptosis assay or the percent viability assay described in Wiley *et al.*, or the cell death assay of Idziorek *et al.* (*See Example 6*), it will be possible to test the effect of TRDL on apoptosis induction or prevention in a variety of cell types, allowing identification of cell types which undergo programmed cell death upon
 35 interaction with TRDL, and of cell types which are prevented from undergoing programmed cell death upon interaction with TRDL.

Therefore, the invention provides a method for the identification of a cell line that undergoes apoptosis upon interaction with TRDL, the method comprising:

- (a) dividing the cells of a culture of a mammalian cell line into a test culture and a control culture;
- 5 (b) contacting a TRDL polypeptide with the test culture of step (a);
- (c) determining the quantity of cells of
 - (i) the test culture obtained in step (b); and
 - (ii) the control culture of step (a);that have undergone apoptosis; and
- 10 (d) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (c)(i) with the quantity of cells determined to have undergone apoptosis in the control culture of step (c)(ii);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is higher than in said control culture indicates that said
15 mammalian cell line undergoes apoptosis upon interaction with TRDL.

A mammalian cell line identified by the above method to undergo apoptosis upon interaction with TRDL may then be used in the identification of agents capable of inhibiting or enhancing TRDL-mediated induction of apoptosis. Therefore, the
20 invention provides a method for the identification of an agent capable of inhibiting TRDL-mediated induction of apoptosis, said method comprising

- (a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises mammalian cells capable of undergoing apoptosis upon interaction with
25 TRDL which have been contacted with a TRDL polypeptide in the presence of a test agent, and said control culture comprises mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent; and
- (b) comparing the quantity of cells determined to have undergone
30 apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is lower than in said control culture indicates that said test agent inhibits TRDL-mediated induction of apoptosis.

35 The invention also provides a method for the identification of an agent capable of enhancing TRDL-mediated induction of apoptosis, said method comprising

- (a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the presence of a test agent, and said control culture comprises mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent; and
- (b) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);
- whereby a determination that the quantity of cells having undergone apoptosis in said test culture is higher than in said control culture indicates that said test agent enhances TRDL-mediated induction of apoptosis.

Another activity exhibited by certain members of the TNF family of cytokines is the prevention of apoptosis. One example may be found in the interaction of tumor necrosis factor with the TNF receptor 2 (TNFR2). That interaction results in the removal of inhibition of NF κ B, a transcription factor which, when activated, results in the protection of the cell from apoptosis.

Therefore, the invention provides a method for the identification of a cell line that is prevented from undergoing apoptosis upon interaction with TRDL, the method comprising:

- (a) dividing the cells of a culture of a mammalian cell line into a test culture and a control culture;
- (b) contacting a TRDL polypeptide with the test culture of step (a);
- (c) determining the quantity of cells of
- (i) the test culture obtained in step (b); and
- (ii) the control culture of step (a);
- that have undergone apoptosis; and
- (d) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (c)(i) with the quantity of cells determined to have undergone apoptosis in the control culture of step (c)(ii);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is lower than in said control culture indicates that said mammalian cell line is prevented from undergoing apoptosis upon interaction with TRDL.

A mammalian cell line identified by the above method to be prevented from

undergoing apoptosis upon interaction with TRDL may then be used in the identification of agents capable of inhibiting or enhancing TRDL-mediated prevention of apoptosis. Therefore, the invention provides a method for the identification of an agent capable of inhibiting TRDL-mediated prevention of

5 apoptosis, said method comprising:

- (a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide
10 in the presence of a test agent, and said control culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent; and
- (b) comparing the quantity of cells determined to have undergone
15 apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is higher than in said control culture indicates that said test agent inhibits TRDL-mediated prevention of apoptosis.

20 The invention also provides the invention provides a method for the identification of an agent capable of enhancing TRDL-mediated prevention of apoptosis, said method comprising

- (a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises
25 mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the presence of a test agent, and said control culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide
30 in the absence of a test agent; and
- (b) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);

whereby a determination that the quantity of cells having undergone apoptosis in
35 said test culture is lower than in said control culture indicates that said test agent enhances TRDL-mediated prevention of apoptosis.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

5 EXAMPLES

Example 1: Isolation and sequencing of a gene encoding TRDL

Materials and Methods

- (a) *BLAST Searches of the Incyte Database:* The Incyte LifeSeq database was searched using the basic local alignment search tool (BLAST) and the complete human TNF- α cDNA sequence (Accession # M10988) as the query (Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Bio.* **215**, 403-410). Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Bio.* **215**, 403-410.
- (b) *Sequence Analysis:* All nucleotide sequences were obtained directly using an ABI373A fluorescence-based sequencer (Perkin Elmer/Applied Biosystems Division, PE/ABD, Foster City, CA) and the ABI PRISM™ Ready Dye-Deoxy Terminator kit with Taq FS™ polymerase. Each ABI cycle sequencing reaction contained about 0.5 μ g of plasmid DNA. Cycle-sequencing was performed using an initial denaturation at 98°C for 1 min, followed by 35 cycles: 96°C for 15 sec, annealing at 50°C for 10 sec, and extension at 60°C for 4 min. Temperature cycles and times were controlled by a Perkin-Elmer 9600 thermocycler. Extension products were purified using Centriscap gel filtration cartridges (Princeton Separation Systems, Adelphia, NJ). Each reaction product was loaded by pipette onto the column, which was then centrifuged in a swinging bucket centrifuge (Sorvall model RT6000B table top centrifuge) at 1500 x g for 4 min at room temperature. Column-purified samples were dried under vacuum for about 40 min and then dissolved in 3 μ l of a DNA loading solution (83% deionized formamide, 8.3 mM EDTA, and 1.6 mg/ml Blue Dextran). The samples were then heated to 90°C for three min and loaded into the gel sample wells for sequence analysis by the ABI373A sequencer (stretch modification). Sequence analysis was done by importing ABI373A files into the Sequencer program (Gene Codes, Ann Arbor, MI). Generally, sequence reads of 700 bp were obtained. Potential sequencing errors were minimized by obtaining sequence information from both DNA strands and by re-sequencing difficult areas using primers at different locations until all sequencing ambiguities were removed. The above methods, along with the LifeSeq Assembly function for the clones in

cluster 99092, including clone 78258, allowed a contiguous 709 bp cDNA sequence to be obtained (Figure 2). Included in the sequence is clone 798247, which lies furthest upstream in the contig.

5 **Results and Discussion**

A BLAST search of the Incyte LifeSeq long-read database using the entire protein coding region of human TNF α (Accession # M10988) as the query identified 7 matches of statistical relevance 30 ($P < 1.0$) (12). Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Bio.* 215, 403-410. Of these matches, 78258R1 displayed homology with TNF α in regions where TNF α was also homologous with FasL (Fas ligand) and TRAIL (TNF-related apoptosis-inducing ligand) (Figures 1A and 1B). A BLAST search of the entire Incyte LifeSeq database using clone 78258R1 identified 23 matches of statistical relevance ($P < 1.0$) (Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Bio.* 215, 403-410), 8 of which were identified as human CpG island DNA genomic MseI fragments. Analysis of the 23 matches revealed that 16 belonged to the same cluster as clone 78258. Assembly of cluster 99092, which contains 78258, produced a contiguous 709 base pair cDNA sequence. This contig was made up of several overlapping clones, including 798247 (from cDNA library OVARNOT03, nontumorous ovarian tissue) which appeared furthest upstream (Figure 2), and retained homology with TNF α , FasL & TRAIL. The 798247 EST sequence available was limited to 285 bp and included a stop codon at the 3' end, but lacked a putative initiation methionine. In order to confirm 798247 as a TNF α homolog, the clone was obtained from Incyte for complete sequence analysis.

Sequence analysis of 798247 in pSPORT (Bethesda Research Laboratories, Bethesda, MD) identified an insert of 828 bp that contained a partial open reading frame of 172 amino acids. Although this clone retained the regions of homology with TNF α , the sequence for 798247 lacked a putative start methionine at the 5' end. This observation necessitated additional cloning experiments to obtain a full length clone and complete characterization.

Two full length clones for 798247 were obtained by screening 5×10^5 plaques from a leukocyte cDNA library. Primary screening identified 5 plaques that hybridized on duplicate lifts. Four of the five were subsequently plaque purified and insert sizes determined by PCR. Insert sizes ranged between 1.5 and 1.8 kb. Sequence analysis of 2 of the clones, TRDL-14 and TRDL-11, in pDR2 (Clontech,

Palo Alto, CA) identified one insert of 1408 bp that contained a putative open reading frame of 248 amino acids, and another insert of 1537 bp that contained a putative open reading frame of 235 amino acids. Clones 14 and 11 were nearly identical, with the exception of a 48 base pair in-frame insertion that encodes sixteen amino acids (residues 111-127) present in clone 14 which is absent from clone 11. Sequence analysis of clones #11 and #14 revealed several differences from the 798247 sequence that appear to arise from alternative splicing of the TRDL gene. Furthermore, an exon of 183 base pairs was present at the 3' end of clone 11 which did not exist in clone 14. Both clones shared near identity with the original clone 798247, and with a contig assembled from Incyte clusters 99092 and 51748. Figure 6 shows the protein alignment of clones TRDL-14 and TRDL-11 with TNF family members.

Another BLAST search against the full length sequence of clone TRDL-14 revealed a second cluster of clones which overlapped the 5' end of the original contig. When assembled, this cluster (51748) produced a 670 bp contig made up of 39 clones. When the 2 clusters were forced into a single assembly, the 3' end of the contig of cluster 51748 overlapped with the 5' end of the contig of cluster 99092, forming a new contig (881955) of 1136 base pairs, and providing an N-terminus which was missing from the original contig.

TRDL shares limited sequence homology with other TNF-related ligands. Sequence conservation with molecules like TNF, TRAIL and FasL was confined primarily to two short stretches within 100 amino acids from the carboxy terminus. The identification of these short, C-terminal stretches of identity allowed selection of the original BLAST match as a possible candidate despite poor statistical scores. The overall percent identity of the C-terminal domain of TRDL to TNF α , FasL and TRAIL is 17%, 17% and 12%, respectively. Although not striking, this level of homology is typical of the TNF cytokine family. By comparison, TRAIL is only 28% homologous to FasL, 23% homologous to TNF α and 22% homologous to lymphotoxin β . Like these family members, TRDL contains a putative transmembrane segment near the N-terminus that is likely required for proper presentation of this molecule on cell surfaces (Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C-P., Nicholl, J.K., Sutherland, G.R., Davis Smith, T., Rauch, C., Smith, C.A., and Goodwin, R.G. (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity*. 3, 673-682).

Example 2: Tissue Distribution of TRDL Expression**Material and Methods**

(a) *Preparation of pDR2/TRDL*: The conversion from a λ DR2 to a 5 pDR2 plasmid was carried out in AM1 cells as described in Clontech Protocol #PT1011-1.

5 Plasmid DNA was purified by Qiagen mini- or maxi-prep (Qiagen, Chatsworth, CA).

(b) *Northern Analysis*: An Incyte Electronic Northern was run on clones 798247 (from cluster 99092) and 177393 (from cluster 51748). This function displays an Electronic Northern and has two objectives: to determine the libraries in which a given gene is expressed and its abundance levels in each one. In the LifeSeq
10 database, this analysis is based on master cluster membership. Clone 177393 was selected because its sequence represents the 5' end of pDR2/TRDL-14.

Multiple human tissue mRNA blots were purchased from Clontech Laboratories (Palo Alto, CA). Plasmid DNA (pSPORT/798247) was digested with *NotI* + *SalI*, purified by QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA), and labeled with
15 $\alpha^{32}\text{P}$ dCTP using a multiprime labeling kit (Ready-to-go DNA labeling kit, Pharmacia Biotech). The blot was hybridized at 68°C for 2.5 h in Express Hyb buffer (Clontech, Palo Alto, CA). Unbound probe was removed by 3 quick rinses then two 20 min washes at room temperature in 2X SSC, 0.05% SDS, followed by two 30 min washes at 50°C in 0.1 X SSC, 0.1% SDS. Hyperfilm MP (Amersham) was exposed
20 overnight at -80°C.

A human cancer cell line multiple tissue mRNA blot was purchased from Clontech (Palo Alto, CA). The first approximately 700 bp of the coding region of pDR2/TRDL-14 were PCR amplified using primers which were 5'-ATATGGATCCC-
AGCTCATGCCAGCCTCA-3' (SEQ ID NO:12) and 5'-AGTAAAGCTTGGAATTATG-
25 ACACTCAGAATATCCC-3' (SEQ ID NO:13). The gel-purified PCR product was labeled for use as a probe, and the blot was hybridized as described above.

(c) *Dot Blot Analysis*: A tumor gene screening 96 dot total RNA dot blot was purchased from Biochain Institute, Inc (San Leandro, CA). The blot was hybridized concomitantly with the cancer cell line northern blot.

30 (d) *Screening of Leukocyte Library*: Screening of Clontech human leukocyte library HL1169X (λ DR2) was carried out as described in the Clontech Lambda Library Protocol Handbook (PR92192). Labeled 798247 probe was produced using Ready-to-go Beads (Pharmacia Biotech) and $\alpha^{32}\text{P}$ dCTP (Amersham). Primary screening of 5×10^5 plaques identified 5 plaques that hybridized on duplicate lifts.
35 Four of the five were plaque purified in 2' and 3' screens, and insert sizes were determined by PCR.

Results and Discussion

Results of Incyte LifeSeq Electronic northern blots for clones 798247 and 177393 are shown in Figure 7. These results indicate that the DNA sequences represented by these 2 clones are expressed mainly in inflammatory tissues, tumors, and tissues with an abundant peripheral blood supply.

The tissue distribution of 798247 was determined by Northern analysis of mRNA isolated from multiple tissues. Hybridization with the full length 798247 insert (cut out of pSPORT w/ *SalI* & *NotI*) identified two RNA species at approximately 1.6 and 1.8 kb. Highest levels of expression were seen in lung and peripheral blood leukocytes, with intermediate levels of expression in pancreas, colon, small intestine, prostate and ovary. There was little or undetectable expression in skeletal muscle, thymus, and testis (Figures 8A and 8B).

A Northern blot of human cancer cell lines was hybridized using the first approximately 700 bp of the coding region of clone TRDL-14. Highly expressed RNA species were identified at 1.7 kb, 2.5 kb in HeLa and SW480 cells. A third species at 4.4 kb was moderately expressed in HeLa, SW480, HL-60, and K-562 cells (Figures 9A and 9B).

A tumor gene screening RNA dot blot was also hybridized using the TRDL-14 probe. Highest levels of expression were seen in tumor samples compared with normal samples of duodenum, colon, and pancreas. By contrast, expression was higher in normal than in tumor samples in tissues such as gallbladder, thyroid, small intestine, thymus, adrenal and breast. Expression in lung was high in both normal and tumor samples (Figures 10A and 10B).

Evidence for alternative splicing was seen via northern analysis using RNA isolated from multiple human tissues. Lung and peripheral blood leukocytes showed high levels of expression of a 1.6 kb transcript only. In contrast, a low level, 1.8 kb transcript appeared in tissues like kidney, pancreas and prostate. This size difference is consistent with the 183 bp exon missing from clone pDR2/TRDL-14, and may correspond to the two transcript sizes seen in tissues. Two transcripts were also seen in northern analyses of tumor cell lines. In this case, both transcripts were present in the same cell lines. Thus, tissues with the highest expression levels (lung and peripheral blood leukocytes) appear to selectively generate the 1.6 kb transcript. In contrast, tissues with low level of TRDL expression produce the 1.8 kb message. This differential expression suggests alternative splicing as a mechanism for tissue-specific expression and functional regulation.

The expression profile of TRDL was similar but not identical to the

expression profile of other TNF-related cytokines. The recently described TRAIL ligand, for example, showed levels of expression in lung and peripheral blood leukocytes that were similar to that seen in other tissues (Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C-P., Nicholl, J.K., Sutherland, G.R., Davis Smith, T., Rauch, C., Smith, C.A., and Goodwin, R.G. (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity*. 3, 673-682). In cancer cell lines, expression of TRDL was primarily limited to HeLa (cervical cancer) and SW480 (colorectal cancer) cell lines, again pointing to a potential for cell specific expression of this gene. Electronic northern analysis of TRDL using the Incyte database revealed an expression pattern that adds to the interest in this molecule as an inflammatory mediator. At least one EST corresponding to TRDL was identified in 42 different libraries. Of these, 24 ESTs appeared in inflamed tissues or tumors. This confirms the presence of TRDL in target disease states.

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Example 3: Heterologous expression of TRDL

Materials and Methods

(a) *pcDNA3/TRDL-14 Expression in E. coli*: The polymerase chain reaction was utilized to amplify the full length coding region of TRDL-14 from pDR2/TRDL-14 using *Bam*HI and *Eco*RI modified primers. The primers used were 5'-CAGCTCATGCCAGCCTCA-3' (SEQ ID NO:14) and 5'-TATCCGTAAAATCAAAGTCCCAG-3' (SEQ ID NO: 15), purchased from Genosys Biotechnologies, Inc. (The Woodlands, TX). 10 ng of plasmid served as the template under the following PCR conditions: 30 cycles (30 sec at 94°C; 1 min at 55°C; 1 min at 72°C). The product was subcloned using a SureClone kit (Pharmacia Biotech). DH5α competent cells (GIBCO-BRL) were transformed and plasmid DNA was purified (Qiagen, Chatsworth CA). The insert was removed by *Bam*HI and *Eco*RI digestion and transferred to pcDNA3 (Invitrogen, Carlsbad CA). DH5α competent cells were transformed and positive colonies determined by PCR analysis. Plasmid DNA from positive colonies was purified (Qiagen) and insert sequence confirmed using an ABI373A fluorescence based sequencer as described above.

(b) *Expression of Human TRDL in CHO cells*: The complete open-reading frame of human TRDL was expressed in Chinese hamster ovary (CHO) cells under the control of the CMV immediate early promotor. Plasmid pDR2/TRDL14 was double digested with *Xba*I and *Bam*HI and the resulting cDNA fragment was ligated into *Xba*I and *Bam*HI digested pUC18 to yield pUC18-TRDL14. pUC18/TRDL14

was the double digested with *EcoRI* and *BamHI* and the resulting DNA fragment was ligated to *EcoRI* and *BamHI* digested pcDNA3 to yield pcDNA3/TRDL14. pcDNA3/TRDL14 was then used to transfect CHO K1 cells using Lipofectamine and stable transfectants were isolated following neomycin selection (G418 0.8 µg/ml).

- 5 Clonal cell lines were prepared by limiting dilution.

The predicted extracellular domains of human TRDL-11 and human TRDL-14 were engineered by PCR for expression as NH₂-terminal fusions with a 6 His-tag in the baculovirus transfer vector pAcHLT-A. A common 5' sense oligonucleotide primer (5'-CATATGCACAGAGCTGCAGAGCCTCAGGAG-3' (SEQ ID NO:16), base
10 pairs 217-239 in TRDL-14 and 226-248 in TRDL-11) containing a *NdeI* restriction site was paired with 3' antisense primers specific for either TRDL-11 or TRDL-14 and containing *KpnI* sites in the PCR (5'-TCACAGTTTTCACAAACCCAGGAAG-3' (SEQ ID NO:17) and 5'-GTAAAATCAAAGTCCCAGGAAGGT-3' (SEQ ID NO:18),
15 respectively). pDR2/TRDL14 plasmid DNA or pDR2/TRDL11 plasmid DNA (0.1 µg) was amplified using the sense and antisense primers (1µM) and the *Pwo* thermostable polymerase under the following cycle conditions; denature for 1 min at 94 °C, anneal for 2 min at 65 °C, and extend for 3 min at 72 °C for 20 cycles. The resulting products were digested to completion with *NdeI* and *KpnI* and ligated into
20 *NdeI* and *KpnI* digested baculovirus transfer vector pAcHLT-A to yield pAcHLT-A/TRDL14 and pAcHLT-A/TRDL11. Both expression plasmids were completely sequenced on both strands to confirm the integrity of the open-reading frame.

(c) *Expression of Human TRDL in baculovirus:* Baculovirus transfer vectors
25 containing either human TRDL-11 (pAcHLT-A/TRDL11) or human TRDL-14 (pAcHLT-A/TRDL14) were co-transfected with BaculoGold™ DNA into Sf9 cells using standard transfection procedures. Intracellular expression was confirmed by metabolic labeling of the cells during infection followed by SDS-PAGE analysis. Five distinct virus plaques were cloned and analyzed for TRDL expression to obtain a
30 single high producing viral isolate. An amplified virus stock then used to infect approximately 1 X 10⁹ cells in Grace's serum-free medium/ liter of either Sf9 cells or Hi5 cells with 5 PFU/cell. Following 65 hr in shaker flasks, the infected cells were harvested by centrifugation and the cell lysate served as the starting material for purification using Ni-agarose.

35 (d) *Engineering of the extracellular domain for secretion from sf9 cells:* The predicted extracellular domain of human TRDL11 and human TRDL14 was

engineered for secretion from sf9 cells by preparing an in-frame fusion with the honey bee melatin leader sequence in the baculovirus transfer vector pVT-Bac (Tessier, D.C., Thomas, D.Y., Khouri, H.E., Laliberte, F., and Vernet, T., *Gene* 98:177-183 (1991)). Sense and antisense PCR primers, incorporating restriction sites for directional cloning into pVT-Bac, were used to amplify the region of the TRDL11 or TRDL14 coding sequences corresponding to the extracellular domain as indicated above. The amplification products were then digested to completion with the appropriate restriction endonucleases and subcloned into pVT-Bac. Expression of pVT-Bac/TRDL11 and pVT-Bac/TRDL14 by cotransfection with BaculoGold DNA was performed as described above.

(e) *Isolation of human TRDL genomic clones:* A partial cDNA clone of human TRDL was used to isolate bacterial artificial chromosome (BAC) genomic clones by colony hybridization. The cDNA insert was random prime labeled with α -³²P-dATP and used to screen a human BAC genomic library. Individual clones were isolated by dilution plating and rescreening and two clones were obtained, 14814 and 14815. PCR analysis using oligonucleotide primers specific for either the shared exon or the TRDL-14/TRDL-11 exons confirmed that both BAC clones contain the entire open-reading frame of human TRDL.

Example 4: Human Chromosomal Mapping of Human TRDL by Fluorescence in situ Hybridization

DNA from clone 14815 was labeled with digoxigenin dUTP by nick translation. The labeled probe was combined with sheared human DNA and hybridized to normal metaphase chromosomes derived from phytohemagglutinin (PHA) -stimulated peripheral blood lymphocytes in a solution containing 50% formamide, 10% dextran sulfate and 2X SSC. Specific hybridization signals were detected by incubation with fluoresceinated antidigoxigenin antibodies followed by counterstaining with 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) for one color experiments. Probe detection in two color experiments was accomplished by incubation with fluoresceinated antidigoxigenin antibodies and Texas red avidin followed by counterstaining with DAPI. The initial experiment resulted in specific labeling of the short arm of a group E chromosome which was believed to be chromosome 17 on the basis of size, morphology and banding pattern. A second experiment was conducted in which a biotin labeled probe specific for the centromeric region of chromosome 17 was cohybridized with clone 14815. This experiment resulted in the specific labeling of the centromer in red and the short

arm of chromosome 17 in green. Measurements of 10 specifically labeled chromosomes 17 demonstrated the 14815 is located at a position which is 77% of the distance from the centromere to the telomere of chromosome arm 17p in an area which corresponds to band 17p13.3. A total of 80 metaphase cells were
5 analyzed with 72 exhibiting specific labeling.

Hypermethylation of CpG islands located within the upstream sequences of vertebrate genes has potential gene silencing consequences. Several studies have demonstrated hypermethylation of CpG islands on human Chromosome 17p13.3 in tumor DNA compared to normal DNA in both colon, breast, and prostate (Ribieras *et al.*, *J. of Cellular Biochemistry* 56:86-96 (1994); Morton, R.A., *et al.*, *J. Urology* 156:512-516 (1996)). An essential role for NF- κ B in preventing TNF- α -induced cell death. *Science*. 274: 782-784). Furthermore, CpG islands within the TRDL gene have led to the annotation of some TRDL ESTs as CpG islands. Thus, it is possible that hypermethylation and consequent inactivation of the TRDL gene results in cancer.

15

Example 5: High Through-Put Screening for TRDL Agonists and Antagonists
Materials and Methods

(a) *Preparation of radiolabeled human TRDL*: The soluble extracellular domain of human TRDL is expressed as an amino terminal 6-His-tagged fusion using
20 baculovirus as described above. Material produced by the infected cells is recovered from the cells by hypotonic lysis/homogenization followed by affinity chromatography on a Ni-NTA agarose. The purified protein is then eluted from the affinity resin using imidazole containing buffer and stored frozen in aliquots prior to use.

A sample of the purified TRDL protein radiolabeled with ^{125}I using iodogen.
25 Iodogen (10 μl of a 1.0 mg/ml solution in CHCl_3) is evaporated under nitrogen, mixed with 30 μl of 1.0 M KPO_4 (pH 7.0)/10 μl (1.0 mCi) of Na^{125}I and incubated for 10 minutes on ice. This mixture is transferred to a tube containing 10 μg of human TRDL in 20 μl and reacted for 5 minutes on ice. Unincorporated Na^{125}I is removed using a PD10 column equilibrated with PBS/0.1% gelatin.

30 (b) *Development of a receptor binding assay and high through-put screening*: ^{125}I -TRDL is used to survey human cell lines for the presence of high affinity receptors [U937, THP-1, Jurkat, HeLa and SW480]. Triplicate tubes containing 1.0×10^6 cells are incubated with 2.0×10^5 dpm of ^{125}I -TRDL in the absence or presence of increasing amounts of unlabeled TRDL over the range 0-10 nM in a total volume
35 of 0.5 ml RPMI 1640 medium/2% FCS. After 2 hr on ice, the cells are washed 3 X with 10 ml fresh medium and cell associated TRDL are quantified by γ -counting.

High through-put screening is done using the scintillation proximity assay (SPA). Membranes prepared from the cell line expressing the highest level of TRDL receptors are coated onto SPA beads by incubation on ice for 1 hour. Membrane-coated SPA beads are mixed with ^{125}I -TRDL and incubated for 30 minutes at room temperature. The SPA beads are then washed with PBS and scintillation detected using a scintillation spectrometer. For screening, the SPA binding assay are performed in the presence or absence of test compounds (10 μM), and agents that reduce the SPA signal by > 50% under these conditions are scored as positive.

10 ***Example 6: Assay for the Quantitation of Cell Death***

Cell death assay: Cell death is quantitated using the DNA binding dye, YOPRO-1 (Molecular Probes, Junction City, OR.) (Idziorek, T., *et al.*, *J. Immuno. Meth.* 185:249-258 (1995). Confluent, serum-starved MCF7 cells in 24-well plates are treated with either UV or etoposide for 16 hours. 1 μM YOPRO-1 is then incubated with the cells at 37°C for 60 minutes. YOPRO-1 uptake is assessed by reading the plate in the fluorescent plate reader at 460 nm excitation and 585 nm emission.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

The entire disclosure of all publications cited herein are hereby incorporated by reference.

What is claimed is:

1. An isolated nucleic acid molecule comprising polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of:
 - 5 (a) a nucleotide sequence encoding a TRDL polypeptide having the complete amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4;
 - (b) a nucleotide sequence encoding the extracellular region of a TRDL polypeptide having the amino acid sequence at about position 53 to about position 235 of SEQ ID NO:2, or about position 53 to about position 248 of SEQ ID NO:4;
 - 10 and
 - (c) a nucleotide sequence complementary to the nucleotide sequence of (a) or (b).
2. The nucleic acid molecule of claim 1, wherein said polynucleotide molecule
15 of 1(a) has the nucleotide sequence given in SEQ ID NO:1 or SEQ ID NO:3.
3. The nucleic acid molecule of claim 1, wherein said polynucleotide molecule of 1(b) has the nucleotide sequence given in SEQ ID NO:1 from about nucleotide 226 to about nucleotide 771, or the nucleotide sequence given in SEQ ID NO:3 from
20 about nucleotide 217 to about nucleotide 801.
4. An isolated nucleic acid molecule comprising polynucleotide which hybridizes under stringent conditions to a polynucleotide having the nucleotide sequence in (a), (b), or (c) of claim 1.
25
5. A vector comprising the nucleic acid molecule of claim 1.
6. The vector of claim 5, wherein said nucleic acid molecule of claim 1 is operably linked to a promoter for the expression of a TRDL polypeptide.
30
7. A host cell comprising the vector of claim 5.
8. A host cell comprising the vector of claim 7.
- 35 9. The host cell of claim 8, wherein said host is a eukaryotic host.

10. The host cell of claim 9, wherein said host cell is a baculovirus cell.
11. The host cell of claim 9, wherein said host cell is a CHO cell.
- 5 12. A method of obtaining a TRDL polypeptide comprising culturing the host cell of claim 8 and isolating said TRDL polypeptide.
13. A TRDL polypeptide produced by the method of claim 12.
- 10 14. An isolated TRDL polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:
- (a) the complete amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4;
 - (b) the amino acid sequence from about position 53 to about position 235 of SEQ ID NO:2; and
 - 15 (c) the amino acid sequence from about position 53 to about position 248 of SEQ ID NO:4.
15. An isolated antibody that binds specifically to the TRDL polypeptide of claim 14.
- 20 16. A method for the identification of cells having a high affinity receptor for TRDL, the method comprising:
- (a) labeling the isolated TRDL polypeptide of claim 14;
 - (b) contacting said labeled TRDL polypeptide obtained in step (a) with
 - 25 cells of a mammalian cell line;
 - (c) washing the cells obtained in step (b) to remove unbound TRDL; and
 - (d) determining the presence of said labeled TRDL polypeptide in the washed cells obtained in step (c);
- whereby the presence of said labeled TRDL polypeptide in the washed cells obtained
- 30 in step (c) indicates the presence of a high affinity receptor for TRDL on said cells.
17. A method for the identification of an agent which inhibits the binding of TRDL to its receptor, said method comprising:
- (a) labeling the isolated TRDL polypeptide of claim 14;
- 35

- (b) contacting said labeled TRDL polypeptide obtained in step (a) with cells of a mammalian cell line
- (i) in the presence of a test agent; and
- (ii) in the absence of a test agent;
- 5 (c) washing the cells
- (i) obtained in step (b)(i); and
- (ii) obtained in step (b)(ii) to remove unbound TRDL;
- (d) determining the amount of labeled TRDL polypeptide in
- (i) the washed cells obtained in step (c)(i); and
- 10 (ii) the washed cells obtained in step (c)(ii); and
- (e) comparing the amount of labeled TRDL polypeptide determined in step (d)(i) to that determined in (d)(ii);

whereby a lower amount of labeled TRDL polypeptide in sample (d)(i) than in sample (d)(ii) indicates that said agent inhibited the binding of TRDL to its receptor.

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18. A method for the identification of an agent which enhances the binding of TRDL to its receptor, said method comprising:

- (a) labeling the isolated TRDL polypeptide of claim 14;
- (b) contacting said labeled TRDL polypeptide obtained in step (a) with
- 20 cells of a mammalian cell line
- (i) in the presence of a test agent; and
- (ii) in the absence of a test agent;
- (c) washing the cells
- (i) obtained in step (b)(i); and
- 25 (ii) obtained in step (b)(ii) to remove unbound TRDL;
- (d) determining the amount of labeled TRDL polypeptide in
- (i) the washed cells obtained in step (c)(i); and
- (ii) the washed cells obtained in step (c)(ii); and
- (e) comparing the amount of labeled TRDL polypeptide determined in
- 30 step (d)(i) to that determined in (d)(ii);

whereby a higher amount of labeled TRDL polypeptide in sample (d)(i) than in sample (d)(ii) indicates that said agent has enhanced the binding of TRDL to its receptor.

- 35 19. A method for the identification of a cell line that undergoes apoptosis upon interaction with TRDL, the method comprising:

- (a) dividing the cells of a culture of a mammalian cell line into a test culture and a control culture;
- (b) contacting a TRDL polypeptide with the test culture of step (a);
- (c) determining the quantity of cells of
 - 5 (i) the test culture obtained in step (b); and
 - (ii) the control culture of step (a);that have undergone apoptosis; and
- (d) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (c)(i) with the quantity of cells
- 10 determined to have undergone apoptosis in the control culture of step (c)(ii);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is higher than in said control culture indicates that said mammalian cell line undergoes apoptosis upon interaction with TRDL.

15

20. A method for the identification of an agent capable of inhibiting TRDL-mediated induction of apoptosis, said method comprising

- (a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises
 - 20 mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the presence of a test agent, and said control culture comprises mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent;
 - 25 and
 - (b) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);
- whereby a determination that the quantity of cells having undergone apoptosis in
- 30 said test culture is lower than in said control culture indicates that said test agent inhibits TRDL-mediated induction of apoptosis.

21. A method for the identification of an agent capable of enhancing TRDL-mediated induction of apoptosis, said method comprising

- 35 (a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises

mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the presence of a test agent, and said control culture comprises mammalian cells capable of undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent; and

(b) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is higher than in said control culture indicates that said test agent enhances TRDL-mediated induction of apoptosis.

22. A method for the identification of an agent capable of inhibiting TRDL-mediated prevention of apoptosis, said method comprising:

(a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the presence of a test agent, and said control culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent; and

(b) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);

whereby a determination that the quantity of cells having undergone apoptosis in said test culture is higher than in said control culture indicates that said test agent inhibits TRDL-mediated prevention of apoptosis.

23. A method for the identification of an agent capable of enhancing TRDL-mediated prevention of apoptosis, said method comprising

(a) determining the quantity of cells that have undergone apoptosis in a test culture and a control culture, wherein said test culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide

in the presence of a test agent, and said control culture comprises mammalian cells that are prevented from undergoing apoptosis upon interaction with TRDL which have been contacted with a TRDL polypeptide in the absence of a test agent; and

- 5 (b) comparing the quantity of cells determined to have undergone apoptosis in the test culture of step (a) with the quantity of cells determined to have undergone apoptosis in the control culture of step (a);
whereby a determination that the quantity of cells having undergone apoptosis in
said test culture is lower than in said control culture indicates that said test agent
10 enhances TRDL-mediated prevention of apoptosis.

Figure 1A

TNF α : 191 YEPIYLGGVFQLEKGDRLSAEINR 214
 | | | | | | + | | | | |
78258: 158 YNXCYXAGVFHLHQGDILSVIIPR 229

Figure 1B

TNF α : 126 VPSEGLYLIYSQVLFK 141
 + | + | | + | | | | +
78258: 5 IQDAGVYLLYRQVLFQ 52

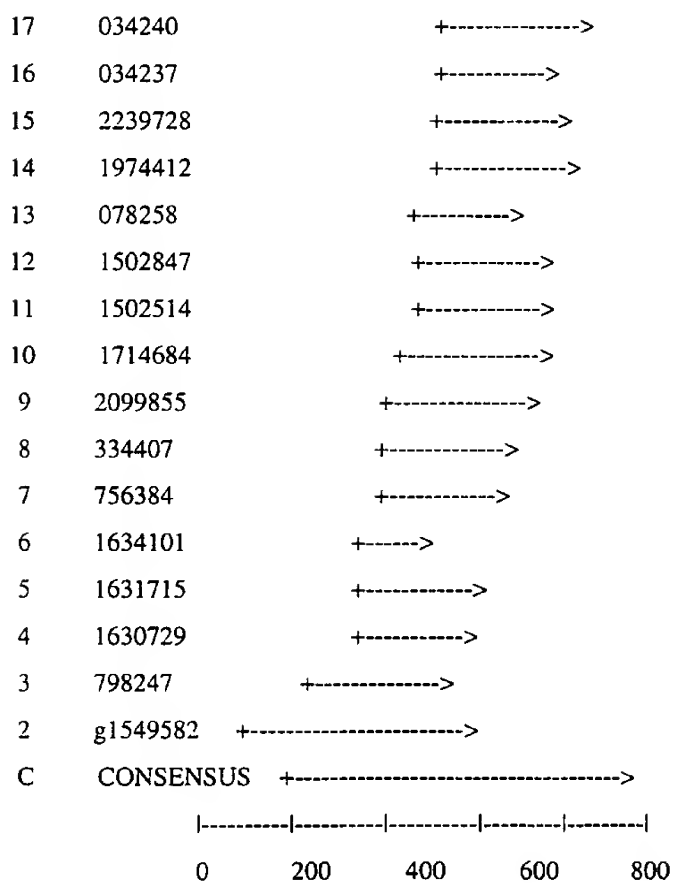
Figure 2

Figure 3

TTTCAAGTCCCGGATCCGCGCTTGCTACCCCACTCTTGAAACCACAGCTGTTGGCAGGGT
 1 CCCCAGCTCATGCCAGCCTCATCTCCTTTCTTGCTAGCCCCCAAAGGGCCTCCAGGCAAC 17
 M P A S S P F L L A P K G P P G N
 18 ATGGGGGGCCAGTCAGAGAGCCGGCACTCTCAGTTGCCCTCTGGTTGAGTTGGGGGGCA 37
 M G G P V R E P A L S V A L W L S W G A
 38 GCTCTGGGGGCGGTGGCTTGTGCCATGGCTCTGCTGACCCAAACAGAGCTGCAGAGC 57
 A L G A V A C A M A L L T Q Q T E L Q S
 58 CTCAGGAGAGAGGTGAGCCGGCTGCAGGGGACAGGAGGCCCTCCAGAATGGGGAAGGG 77
 L R R E V S R L Q G T G G P S Q N G E G
 78 TATCCCTGGCAGAGTCTCCCGGAGCAGAGTTCCGATGCCCTGGAAGCCTGGGAGAGTGGG 97
 Y P W Q S L P E Q S S D A L E A W E S G
 98 GAGAGATCCCGAAAAGGAGAGCAGTGCTCACCACAAAACAGAAGAATGACTCCGATGTG 117
 E R S R K R R A V L T Q K Q K N D S D V
 118 ACAGAGGTGATGTGGCAACCAGCTCTTAGGCGTGGGAGAGGCCTACAGGCCAAGGATAT 137
 T E V M W Q P A L R R G R G L Q A Q G Y
 138 GGTGTCCGAATCCAGGATGCTGGAGTTTATCTGCTGTATAGCCAGGTCCTGTTCAAGAC 157
 G V R I Q D A G V Y L L Y S Q V L F Q D
 158 GTGACTTTCACCATGGGTGAGTGTCTCGAGAAGGCCAAGGAAGGCAGGAGACTCTA 177
 V T F T M G Q V V S R E G Q G R Q B T L
 178 TTCCGATGTATAAGAAGTATGCCCTCCACCCGGACCGGGCCTACAACAGCTGCTATAGC 197
 F R C I R S M P S H P D R A Y N S C Y S
 198 GCAGGTGCTTCCATTTACACCAAGGGGATATTCTGAGTGTGATAATTCCCCGGGCAAGG 217
 A G V F H L H Q G D I L S V I I P R A R
 218 GCGAACTTAACCTCTCTCCACATGGAACCTTCTGGGGTTTGTGAACTGTGATTGTGT 234
 A K L N L S P H G T F L G F V K L
 TATAAAAGTGGCTCCCAGCTTGAAGACCAGGGTGGGTACATACTGGAGACAGCCAAGA
 GCTGAGTATATAAAGGAGAGGGAATGTGCAGGAACAGAGGCGTCTTCTGGGTTTGGCTC
 CCCGTTCTCACTTTTCCCTTTTCACTCCACCCCTAGACTTTGATTTTACGGATATCT
 TGCTTCTGTTCCCATGGAGCTCCGAATTCTTGGTGTGTGTAGATGAGGGGCGGGGACG
 GGGCCAGGCATTGTCCAGACCTGGTCCGGCCCACTGGAAGCATCCAGAACAGCACCACC
 ATCTAGCGGCGCTCGAGGGAAGCACCCGCGGTGGCCGAAGTCCAGGAAGCCGCCCTC
 TGCTAGGGAACCCCTGTTCTCCATGCCACCTCTCTCCAGGTGCCCTCTGCTCTT
 CACCCACAGAAGCCTTATCTACGTCTTCTCTCATCTATCGGACCCCACTTTCCAT
 CACTATCTCCAGAGATGTAGCTATTATGCGCCCGTCTACAGGGGGTGGCCGACGATGACG
 GTGCTTCCAGTCAAATTACTCTCGGGTCCCAAGTTTGGCTTTTACGGCTCCATTG
 CCCCCGCGTGGCAGGCCATTCCAAGCCCTCCGGGCTGGAAGTGGTGTGGAGGAGCCTC
 GGGTGTATCGTACGCCCTGGTGTGGTGTGCTCACTCCTCTGAGCTCTTCTTCTGAT
 CAAGCCCTGCTTAAAGTTAAATAAATAAGAATGAATGATAAAAAAAAAAAAAA

Figure 4

TTTTATTTAGGTCCCGGATCCGCGCTTGAAACCACAGCTGTTGGCAGGGTCCCCAGCTC
1 ATGCCAGCCTCATCTCCTTTCTTGCTAGCCCCCAAAGGGCCTCCAGGCAACATGGGGGGC 20
M P A S S P F L L A P K G P P G N M G G
21 CCAGTCAGAGAGCCGGCACTCTCAGTTGCCCTCTGGTTGAGTTGGGGGGCAGCTCTGGGG 40
P V R E P A L S V A L W L S W G A A L G
41 GCCGTGGCTTGTGCCATGGCTCTGCTGACCCAAACAGAGCTGCAGAGCCTCAGGAGA 60
A V A C A M A L L T Q Q T E L Q S L R R
61 GAGGTGAGCCGGCTGCAGGGGACAGGAGGCCCTCCAGAATGGGGAAGGGTATCCCTGG 80
E V S R L Q G T G G P S Q N G E G Y P W
81 CAGAGTCTCCCGGAGCAGAGTTCCGATGCCCTGGAAGCCTGGGAGAATGGGAGAGATCC 100
Q S L P E Q S S D A L E A W E N G E R S
101 CGGAAAAGGAGAGCAGTGCTCACCACAAAACAGAAGAAGCAGCACTCTGCTCCTGCACCTG 120
R K R R A V L T Q K Q K K Q H S V L H L
121 GTTCCCATTAACGCCACCTCCAAGGATGACTCCGATGTGACAGAGGTGATGTGGCAACCA 140
V P I N A T S K D D S D V T E V M W Q P
141 GCTCTTAGCGGTGGGAGAGGCCTACAGGCCAAGGATATGGTGTCCGAATCCAGGATGCT 160
A L R R G R G L Q A Q G Y G V R I Q D A
161 GGAGTTTATCTGCTGTATAGCCAGGTCCTGTTTCAAGACGTGACTTTCACCATGGGTCAG 180
G V Y L L Y S Q V L F Q D V T F T M G Q
181 GTGGTGTCTCGAGAAGGCCAAGGAAGGCAGGAGACTCTATTCCGATGTATAAGAAGTATG 200
V V S R E G Q G R Q E T L F R C I R S M
201 CCCTCCACCCGGACCGGGCCTACAACAGCTGCTATAGCGCAGGTGTCTTCCATTACAC 220
P S H P D R A Y N S C Y S A G V F H L H
221 CAAGGGGATATTCTGAGTGTCTATAATTCCCGGGCAAGGGCGAACTTAACCTCTCTCCA 240
Q G D I L S V I I P R A R A K L N L S P
241 CATGGAACCTTCTGGGACTTTGATTTTACGGATATCTTGCTTCTGTTCCCCATGGAGCT 247
H G T F L G L
CCGAATTCTTGCGTGTGTGTAGATGAGGGGCGGGGACGGGCGCCAGGCATTGTTTCAGAC
CTGGTCGGGGCCCACTGGAAGCATCCAGAACAGCACCACCATCTAGCGGCCGCTCGAGGG
AAGCACCCGCGGTTGGCCGAAGTCACGAAGCCGCCCCTCTGCTAGGGAAAACCCCTGGT
TCTCCATGCCACACCTCTCTCCAGGTGCCCTCTGCCTCTTACCCCAAGAAGCCCTAT
CCTACGTCCTTCTCTCCATCTATCGGACCCCAAGTTCCATCACTATCTCCAGAGATGTAG
CTATTATGCGCCCGTCTACAGGGGGTCCCCGACGATGACGGTGCCTTCGCAGTCAAATTA
CTCTTCGGGTCCCAAGGTTTGGCTTTCACGCGCTCCATTGCCCCGGCGTGGCAGGCCATT
CCAAGCCCTTCCGGGCTGGAAGTGTGTCGGAGGAGCCTCGGGTGTATCGTACGCCCTGG
TGTTGGTGTGCTCACTCCTCTGAGCTCTTCTTCTGATCAAGCCCTGCTTAAAGTTAA
ATAAAATAGAATGAATGATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 5

```

1 .....TTTCAGGTCCCGGATCCGCGCTTGCTACCCCACTCTTGAAACCAC 45
1 TTTTATTTTTCAGGTCCCGGATCCGCG.....CTTGAAACCAC 36
46 AGCTGTTGGCAGGGTCCCCAGCTCATGCCAGCCTCATCTCCTTTCTTGCT 95
37 AGCTGTTGGCAGGGTCCCCAGCTCATGCCAGCCTCATCTCCTTTCTTGCT 86
96 AGCCCCCAAAGGGCCTCCAGGCAACATGGGGGGCCAGTCAGAGAGCCGG 145
87 AGCCCCCAAAGGGCCTCCAGGCAACATGGGGGGCCAGTCAGAGAGCCGG 136
146 CACTCTCAGTTGCCCTCTGGTTGAGTTGGGGGGCAGCTCTGGGGGCCGTG 195
137 CACTCTCAGTTGCCCTCTGGTTGAGTTGGGGGGCAGCTCTGGGGGCCGTG 186
196 GCTTGTGCCATGGCTCTGCTGACCCAACAACAGAGCTGCAGAGCCTCAG 245
187 GCTTGTGCCATGGCTCTGCTGACCCAACAACAGAGCTGCAGAGCCTCAG 236
246 GAGAGAGGTGAGCCGGCTGCAGGGGACAGGAGGCCCTCCAGAAATGGGG 295
237 GAGAGAGGTGAGCCGGCTGCAGGGGACAGGAGGCCCTCCAGAAATGGGG 286
296 AAGGGTATCCCTGGCAGAGTCTCCCGGAGCAGAGTTCCGATGCCCTGGAA 345
287 AAGGGTATCCCTGGCAGAGTCTCCCGGAGCAGAGTTCCGATGCCCTGGAA 336
346 GCCTGGGAGAGTGGGAGAGATCCCGAAAAGGAGAGCAGTGCTCACCCA 395
337 GCCTGGGAGAAATGGGAGAGATCCCGAAAAGGAGAGCAGTGCTCACCCA 386
396 AAAACAGAAGA..... 406
387 AAAACAGAAGAAGCAGCACTCTGTCTGCACCTGGTTCCCATTAACGCCA 436
407 .....ATGACTCCGATGTGACAGAGGTGATGTGGCAACCAGCTCTT 447
437 CCTCCAAGGATGACTCCGATGTGACAGAGGTGATGTGGCAACCAGCTCTT 486
448 AGGCGTGGGAGAGGCCTACAGGCCCAAGGATATGGTGTCCGAATCCAGGA 497
487 AGGCGTGGGAGAGGCCTACAGGCCCAAGGATATGGTGTCCGAATCCAGGA 536
498 TGCTGGAGTTTATCTGCTGTATAGCCAGGTCTGTTTCAAGACGTGACTT 547
537 TGCTGGAGTTTATCTGCTGTATAGCCAGGTCTGTTTCAAGACGTGACTT 586
548 TCACCATGGGTGAGGTGGTGTCTCGAGAAGGCCAAGGAAGGCAGGAGACT 597
587 TCACCATGGGTGAGGTGGTGTCTCGAGAAGGCCAAGGAAGGCAGGAGACT 636
598 CTATTCCGATGTATAAGAAGTATGCCCTCCACCCGGACCGGGCCTACAA 647
637 CTATTCCGATGTATAAGAAGTATGCCCTCCACCCGGACCGGGCCTACAA 686
648 CAGCTGCTATAGCGCAGGTGTCTTCCATTACACCAAGGGGATATTCTGA 697
687 CAGCTGCTATAGCGCAGGTGTCTTCCATTACACCAAGGGGATATTCTGA 736

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698 GTGTCATAATCCCCGGGCAAGGGCGAACTTAACCTCTCTCCACATGGA 747
|||||
737 GTGTCATAATCCCCGGGCAAGGGCGAACTTAACCTCTCTCCACATGGA 786
748 ACCTTCCTGGGGTTGTGAACTGTGATTGTGTTATAAAAAGTGGCTCCC 797
|||||
787 ACCTTCCTGG..... 796
898 CTCCCCGTTCCTCACTTTTCCCTTTTCATTCCCACCCCTAGACTTTGAT 947
|||||
797GACTTTGAT 805
948 TTTACGGATATCTTGCTTCTGTTCCCATGGAGCTCCGAATTCTTGCGTG 997
|||||
806 TTTACGGATATCTTGCTTCTGTTCCCATGGAGCTCCGAATTCTTGCGTG 855
998 TGTGTAGATGAGGGGC.GGGGACGGGCGCCAGGCATTGTCCAGACCTGGT 1046
|||||
856 TGTGTAGATGAGGGGCGGGGACGGGCGCCAGGCATTGTTCAGACCTGGT 905
1047 C.GGGCCCACTGGAAGCATCCAGAACAGCACCACCATCTAGCGGCCGCTC 1095
|
906 CGGGCCCACTGGAAGCATCCAGAACAGCACCACCATCTAGCGGCCGCTC 955
1096 GAGGGAAGCACC CGCGGTTGGCCGAAGTCCACGAAGCCGCCCTCTGCTA 1145
|||||
956 GAGGGAAGCACC CGCGGTTGGCCGAAGTCCACGAAGCCGCCCTCTGCTA 1005
1146 GGGAAAACCCCTGGTTCTCCATGCCACACCTCTCTCCAGGTGCCCTCTGC 1195
|||||
1006 GGGAAAACCCCTGGTTCTCCATGCCACACCTCTCTCCAGGTGCCCTCTGC 1055
1196 CTCTTCACCCACACAAGAAGCCTTATCCTACGTCCTTCTCTCCATCTATCG 1245
|||||
1056 CTCTTCACCCACACAAGAAGCCTTATCCTACGTCCTTCTCTCCATCTATCG 1105
1246 GACCCCAAGTTTCCATCACTATCTCCAGAGATGTAGCTATTATGCGCCCGT 1295
|||||
1106 GACCCCAAGTTTCCATCACTATCTCCAGAGATGTAGCTATTATGCGCCCGT 1155
1296 CTACAGGGGGTGCCCGACGATGACGGTGCCCTTCGCAGTCAAATTACTCTT 1345
|||||
1156 CTACAGGGGGTGCCCGACGATGACGGTGCCCTTCGCAGTCAAATTACTCTT 1205
1346 CGGGTCCCAAGGTTTGGCTTTCACGCGCTCCATTGCCCCGGCGTGGCAGG 1395
|||||
1206 CGGGTCCCAAGGTTTGGCTTTCACGCGCTCCATTGCCCCGGCGTGGCAGG 1255
1396 CCATTCCAAGCCCTTCCGGGCTGGAAGTGGTGTGCGAGGAGCCTCGGGTG 1445
|||||
1256 CCATTCCAAGCCCTTCCGGGCTGGAAGTGGTGTGCGAGGAGCCTCGGGTG 1305
1446 TATCGTACGCCCTGGTGTTGGTGTGCTCCTCACTCCTCTGAGCTCTTCTT 1495
|||||
1306 TATCGTACGCCCTGGTGTTGGTGTGCTCCTCACTCCTCTGAGCTCTTCTT 1355
1496 CTGATCAAGCCCTGCTTAAAGTTAAATAAATAGAATGAATGATAAAAAA 1545
|||||
1356 CTGATCAAGCCCTGCTTAAAGTTAAATAAATAGAATGAATGATAAAAAA 1405
1546 AAAAAAAA..... 1554

	1		50
TRDL-11
TRDL-14
TNFa
FasL	MQQPFNYYPY	QIYWVDSSAS	SPWAPPGTVL PCPTSVPRRP QRRRPPPPPP
TRAIL
	51		100
TRDL-11MPAS	SPFLLAPKGP	PGNMGGPV.R EPALSVALWL SWGAALGAVA
TRDL-14MPAS	SPFLLAPKGP	PGNMGGPV.R EPALSVALWL SWGAALGAVA
TNFa	.MSTESMIRD	VELAEEALPK	.KTGGPQG.S RRCLFSLFS FLI..VAGAT
FasL	PPPLPPPPPP	PPLPPLPLPP	LKKRGNHS.T GLCLLMFFM VLVALVGLGL
TRAILMAM	MEVQGGPSLG QTCVLIVIFT VLLQSLCAVAV
	101		150
TRDL-11	CAMALLTQQT	ELQS.....LRREVS RLQGTGGPSQ NGEQYPWQSL
TRDL-14	CAMALLTQQT	ELQS.....LRREVS RLQGTGGPSQ NGEQYPWQSL
TNFa	TLFCLLHFGV	IGPQ.....REESPR DLSLISPLAQ AVRS.....
FasL	GMFQLFHLQK	ELAE.....LRESTS QMHTASSLEK QIGH.....
TRAIL	TVYFTNELK	QMOKYKSG	IACFLKEDDS YWDPNEESM NSPCWQVKWQ
	151		200
TRDL-11	PEQSSDALEA	WESGER....SRKRR AVLTKQK...
TRDL-14	PEQSSDALEA	WENGER....SRKRR AVLTKQKQKKQ
TNFaSS	RTPSDK.....PV AHVVAN....
FasLPS	PPPEKK.....ELRKY AHLTKG....
TRAIL	LRQLVRKMIL	RTSEETISTV	QEKQONISPL VRERGPQVA AHITGTRGRS
	201		250
TRDL-11KNDSVDT	EVMWQPALRR GRGLQAQGYG VRIQDAGVYL
TRDL-14	HSVLHLVPIN	ATSKDDSDVT	EVMWQPALRR GRGLQAQGYG VRIQDAGVYL
TNFaPQAEQQLQ	WLNRRANALL ANGVELRDQ LVPVSEGLYL
FasLSNRSRMP	EWEDTYGIVL LSGVKYKKGK LVINETGLYF
TRAIL	NTLSSPNSKN	EKALGRKINS	WESSRSGHSF LSNLHLRNGE LVIHEKGFYF
	251		300
TRDL-11	LYSQVLFQDV	TFTMGQVVS	EGQGR...QE TLFRCIRSMP SHPDRAY...
TRDL-14	LYSQVLFQDV	TFTMGQVVS	EGQGR...QE TLFRCIRSMP SHPDRAY...
TNFa	IYSQVLFKQ	GCPSTHLLT	HTISR...IA VSYQTKVNL SAIKSPCORE
FasL	VYSKVYFRQ	SC..NNLPLS	HKVYM...RN SKYPQDLVMM EGKMSY...
TRAIL	IYSQVYFRQ	EEIKENTKND	KQMVQYIYKY TSYPDPILLM KSARNSCWSK
	301		350
TRDL-11NSCYSAGVF	HLHQGDILSV IIPRARAKLN LSPHGTFLGF
TRDL-14NSCYSAGVF	HLHQGDILSV IIPRARAKLN LSPHGTFLGL
TNFa	TPEGAEAKPW	YEPIYLGGVF	QLEKGDRLSA EINRPDYLDF AESGQVYFGI
FasL	..CTTGQMW	ARSSYLGAVF	NLTSADHLYV NVSELSLVNF EES.QTFFGL
TRAIL	DAEYG.....	LYSIYQGGIF	ELKENDRIFV SVTNEHLID. MDHEASFFGA
	351		
TRDL-11	VKL*		
TRDL-14	*...		
TNFa	IAL.		
FasL	YKL.		
TRAIL	FLVG		

Figure 7

Electronic Northern for Clone: 177393

Library	Lib Description	Abun	Pct	Abun
-----	-----	-----	-----	-----
TYMNOT01	lymphocytes (non-adher PBMNC), 24 M	1	0.1070	
BLADNOT01	bladder, 78 F	3	0.1051	
LUNGNOT14	lung, 47 M	4	0.1037	
THYRNOT02	thyroid, hyperthyroidism, 16 F	2	0.0607	
KIDNTUT13	kidney tumor, carcinoma, 51 F	2	0.0529	
BLADNOT05	bladder, 60 M, match to BLADTUT04	2	0.0528	
COLNNOT23	colon, ulcerative colitis, 16 M	2	0.0528	
LUNGTUT11	lung tumor, carcinoma, 57 M	2	0.0475	
SINIUCT01	small intestine, ileum, 42 M	1	0.0299	
NPOLNOT01	nasal polyp, 78 M	1	0.0286	
UTRSNOT05	uterus, 45 F	1	0.0278	
UTRSNOT11	uterus, myometrium, 43 F	1	0.0277	
SINTNOT13	sm intest, ileum, ulcerative colitis, 25F	1	0.0275	
PROSTUT10	prostate tumor, 66 M, match to PROSNOT15	1	0.0268	
LEUKNOT03	white blood cells, 27 F	1	0.0262	
SINTFET03	small intestine, fetal F	2	0.0259	
PANCTUT01	pancreatic tumor, 65 F, match to PANCNOT08	1	0.0258	
SKINBIT01	skin, leg, erythema nodosum	1	0.0256	
SCORNOT01	spinal cord, 71 M	1	0.0201	
LUNGNOT03	lung, 79 M, match to LUNGTUT02	1	0.0200	
LUNGTUT02	lung tumor, metas, 79 M, match to LUNGNOT03	1	0.0189	
BRSTTUT02	breast tumor, 54 F, match to BRSTNOT03	1	0.0140	
OVARTUT01	ovarian tumor, 43 F, match to OVARNOT03	1	0.0103	
BRSTNOT04	breast, 62 F	1	0.0096	
LUNGA01	lung, asthma, 17 M	1	0.0094	
PROSNON01	prostate, 28 M, NORM	1	0.0094	
BRAITUT02	brain tumor, metastasis, 58 M	1	0.0075	
NGANNOT01	ganglioneuroma, 9 M	1	0.0073	

Electronic Northern for Clone: 798247

Library	Lib Description	Abun	Pct	Abun
-----	-----	-----	-----	-----
COLNNOT19	large intestine, cecum, 18 F	3	0.0878	
THPINOB01	THP-1 promonocyte cell line, control	2	0.0657	
BRAITUT07	brain tumor, left frontal, 32 M	2	0.0517	
PROSNOT02	prostate, 50 M, match to PROSTUT01	1	0.0435	
TONSNOT01	tonsil, hyperplasia, 6 M	1	0.0339	
UCMCNOT02	mononuclear cells	1	0.0236	
SYNORAB01	synovium, hip, rheumatoid, 68 F	1	0.0195	
SYNOOAT01	synovium, knee, osteoarthritis, 82 F	1	0.0180	
OVARNOT03	ovary, 43 F, match to OVARNOT01	1	0.0167	
BRAITUT02	brain tumor, metastasis, 58 M	2	0.0149	
EOSIHET02	eosinophils, hypereosinophilia, 48 M	1	0.0105	
UTRPNOM01	uterus, F, NORM, WM	1	0.0101	
PANCTUT02	pancreatic tumor, carcinoma, 45 F	1	0.0086	
UCMCL5T01	mononuclear cells, treated IL-5	1	0.0084	

Figure 8A

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

- 2.4
- 1.35

Figure 8B

Multiple Tissue Northern

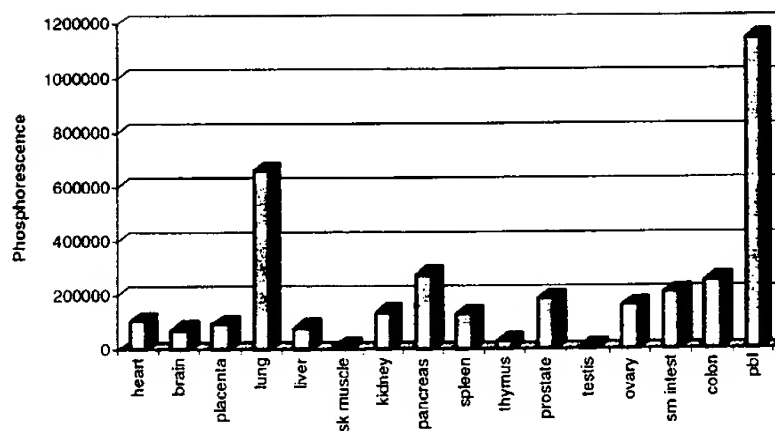


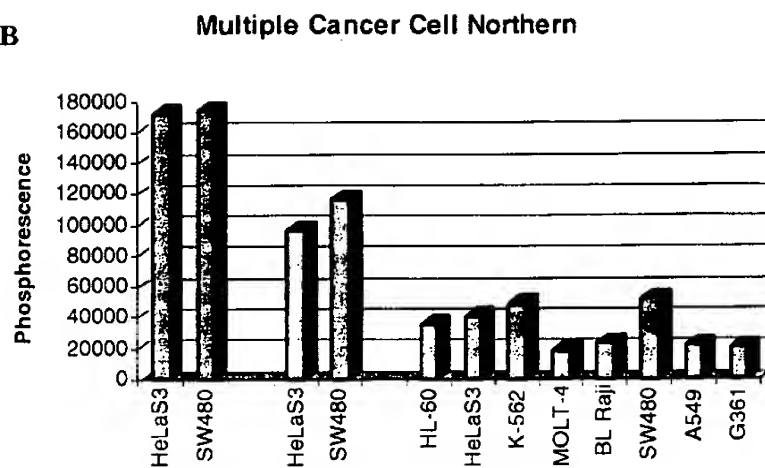
Figure 9A**Figure 9B**

Figure 10A

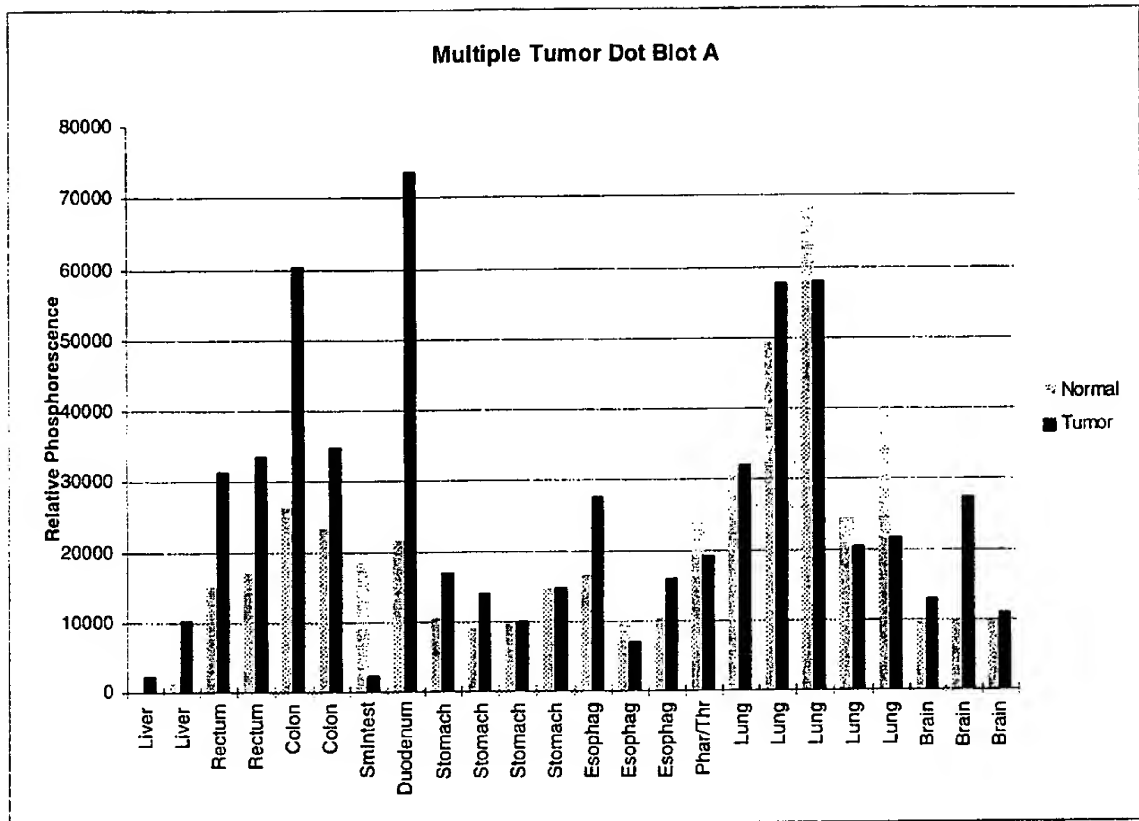
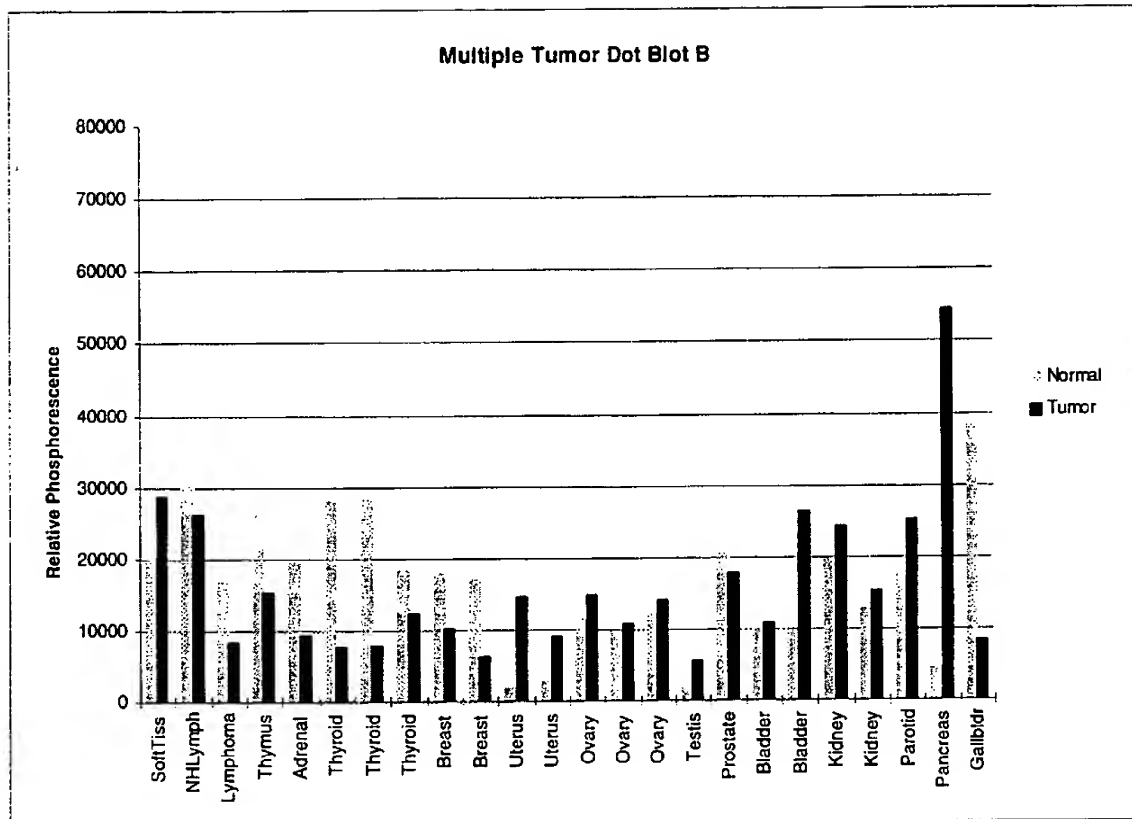


Figure 10 B



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Bienkowski, Michael J
Mills, Cynthia J
Jones, David A

(ii) TITLE OF INVENTION: TNF-Related Death Ligand

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Pharmacia & Upjohn, Intellectual Property
Legal Services
(B) STREET: 301 Henrietta Street
(C) CITY: Kalamazoo
(D) STATE: MI
(E) COUNTRY: USA
(F) ZIP: 49001

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy Disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Kerber, Lori L.
(B) REGISTRATION NUMBER: 41,113
(C) REFERENCE/DOCKET NUMBER: 6111.P CN1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 616/833-0974
(B) TELEFAX: 616/833-8897
(C) TELEX: 224401

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1550 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTTCAGGTCC CGGATCCGCG CTTGCTACCC CACTCTTGAA ACCACAGCTG TTGGCAGGGT	60
CCCCAGCTCA TGCCAGCCTC ATCTCCTTTC TTGCTAGCCC CCAAAGGGCC TCCAGGCAAC	120
ATGGGGGGCC CAGTCAGAGA GCCGGCACTC TCAGTTGCCC TCTGGTTGAG TTGGGGGGCA	180
GCTCTGGGGG CCGTGGCTTG TGCCATGGCT CTGCTGACCC AACAAACAGA GCTGCAGAGC	240
CTCAGGAGAG AGGTGAGCCG GCTGCAGGGG ACAGGAGGCC CCTCCCAGAA TGGGGAAGGG	300
TATCCCTGGC AGAGTCTCCC GGAGCAGAGT TCCGATGCCC TGGAAGCCTG GGAGAGTGGG	360
GAGAGATCCC GGAAAAGGAG AGCAGTGCTC ACCCAAAAAC AGAAGAATGA CTCCGATGTG	420
ACAGAGGTGA TGTGGCAACC AGCTCTTAGG CGTGGGAGAG GCCTACAGGC CCAAGGATAT	480
GGTGTCCGAA TCCAGGATGC TGGAGTTTAT CTGCTGTATA GCCAGGTCCT GTTCAAGAC	540
GTGACTTTCA CCATGGGTCA GGTGGTGTCT CGAGAAGGCC AAGGAAGGCA GGAGACTCTA	600
TTCCGATGTA TAAGAAGTAT GCCCTCCCAC CCGGACCGGG CCTACAACAG CTGCTATAGC	660
GCAGGTGTCT TCCATTTACA CCAAGGGGAT ATTCTGAGTG TCATAATTCC CCGGGCAAGG	720
GCGAAACTTA ACCTCTCTCC ACATGGAACC TTCCTGGGGT TTGTGAACT GTGATTGTGT	780
TATAAAAAGT GGCTCCCAGC TTGGAAGACC AGGGTGGGTA CATACTGGAG ACAGCCAAGA	840
GCTGAGTATA TAAAGGAGAG GGAATGTGCA GGAACAGAGG CGTCTTCCTG GGTTCGGCTC	900
CCCGTTCCTC ACTTTTCCCT TTTCATTCCC ACCCCCTAGA CTTTGATTTT ACGGATATCT	960
TGCTTCTGTT CCCCATGGAG CTCCGAATTC TTGCGTGTGT GTAGATGAGG GGCGGGGACG	1020
GGCGCCAGGC ATTGTCCAGA CCTGGTCGGG CCCACTGGAA GCATCCAGAA CAGCACCACC	1080

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ATCTAGCGGC CGCTCGAGGG AAGCACCCGC CGGTTGGCCG AAGTCCACGA AGCCGCCCTC 1140
TGCTAGGGAA AACCCCTGGT TCTCCATGCC ACACCTCTCT CCAGGTGCCC TCTGCCTCTT 1200
CACCCACAA GAAGCCTTAT CCTACGTCCT TCTCTCCATC TATCGGACCC CAGTTTCCAT 1260
CACTATCTCC AGAGATGTAG CTATTATGCG CCCGTCTACA GGGGGTGCCC GACGATGACG 1320
GTGCCTTCGC AGTCAAATTA CTCTTCGGGT CCCAAGGTTT GGCTTTCACG CGCTCCATTG 1380
CCCCGGCGTG GCAGGCCATT CCAAGCCCTT CCGGGCTGGA ACTGGTGTCT GAGGAGCCTC 1440
GGGTGTATCG TACGCCCTGG TGTGTTGTTT GCCTCACTCC TCTGAGCTCT TCTTTCTGAT 1500
CAAGCCCTGC TTAAAGTTAA ATAAATAGA ATGAATGATA AAAAAAAAAA 1550

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 234 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Pro Ala Ser Ser Pro Phe Leu Leu Ala Pro Lys Gly Pro Pro Gly
1           5           10           15

Asn Met Gly Gly Pro Val Arg Glu Pro Ala Leu Ser Val Ala Leu Trp
          20           25           30

Leu Ser Trp Gly Ala Ala Leu Gly Ala Val Ala Cys Ala Met Ala Leu
          35           40           45

Leu Thr Gln Gln Thr Glu Leu Gln Ser Leu Arg Arg Glu Val Ser Arg
          50           55           60

Leu Gln Gly Thr Gly Gly Pro Ser Gln Asn Gly Glu Gly Tyr Pro Trp
65           70           75           80

Gln Ser Leu Pro Glu Gln Ser Ser Asp Ala Leu Glu Ala Trp Glu Ser
          85           90           95

```


Gly Glu Arg Ser Arg Lys Arg Arg Ala Val Leu Thr Gln Lys Gln Lys
 100 105 110
 Asn Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg Arg
 115 120 125
 Gly Arg Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp Ala
 130 135 140
 Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr Phe
 145 150 155 160
 Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu Thr
 165 170 175
 Leu Phe Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala Tyr
 180 185 190
 Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp Ile
 195 200 205
 Leu Ser Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser Pro
 210 215 220
 His Gly Thr Phe Leu Gly Phe Val Lys Leu
 225 230

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1430 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTTATTTC A GGTCCCGGAT CCGCGCTTGA AACCACAGCT GTTGGCAGGG TCCCCAGCTC 60
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GCCGTGGCTT GTGCCATGGC TCTGCTGACC CAACAAACAG AGCTGCAGAG CCTCAGGAGA 240
 GAGGTGAGCC GGCTGCAGGG GACAGGAGGC CCCTCCCAGA ATGGGGAAGG GTATCCCTGG 300
 CAGAGTCTCC CGGAGCAGAG TTCCGATGCC CTGGAAGCCT GGGAGAATGG GGAGAGATCC 360
 CGGAAAAGGA GAGCAGTGCT CACCCAAAAA CAGAAGAAGC AGCACTCTGT CCTGCACCTG 420
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 GCTCTTAGGC GTGGGAGAGG CCTACAGGCC CAAGGATATG GTGTCCGAAT CCAGGATGCT 540
 GGAGTTTATC TGCTGTATAG CCAGGTCCTG TTTCAAGACG TGACTTTCAC CATGGGTCAG 600
 GTGGTGTCTC GAGAAGGCCA AGGAAGGCAG GAGACTCTAT TCCGATGTAT AAGAAGTATG 660
 CCCTCCCACC CGGACCGGGC CTACAACAGC TGCTATAGCG CAGGTGTCTT CCATTTACAC 720
 CAAGGGGATA TTCTGAGTGT CATAATTCCC CGGGCAAGGG CGAAACTTAA CCTCTCTCCA 780
 CATGGAACCT TCCTGGGACT TTGATTTTAC GGATATCTTG CTTCTGTTCC CCATGGAGCT 840
 CCGAATTCTT GCGTGTGTGT AGATGAGGGG CGGGGGACGG GCGCCAGGCA TTGTTTCAGAC 900
 CTGGTCGGGG CCCACTGGAA GCATCCAGAA CAGCACCACC ATCTAGCGGC CGCTCGAGGG 960
 AAGCACCCGC CGGTTGGCCG AAGTCCACGA AGCCGCCCTC TGCTAGGGAA AACCCCTGGT 1020
 TCTCCATGCC ACACCTCTCT CCAGGTGCCC TCTGCCTCTT CACCCACAA GAAGCCTTAT 1080
 CCTACGTCCT TCTCTCCATC TATCGGACCC CAGTTTCCAT CACTATCTCC AGAGATGTAG 1140
 CTATTATGCG CCCGTCTACA GGGGGTGCCC GACGATGACG GTGCCTTCGC AGTCAAATTA 1200
 CTCTTCGGGT CCCAAGGTTT GGCTTTTCAG CGCTCCATG CCCC GGCGTG GCAGGCCATT 1260
 CCAAGCCCTT CCGGGCTGGA ACTGGTGTG GAGGAGCCTC GGGTGTATCG TACGCCCTGG 1320
 TGTGGTGTT GCCTCACTCC TCTGAGCTCT TCTTTCTGAT CAAGCCCTGC TTAAAGTTAA 1380
 ATAAAATAGA ATGAATGATA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1430

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 247 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Pro Ala Ser Ser Pro Phe Leu Leu Ala Pro Lys Gly Pro Pro Gly
1           5           10           15

Asn Met Gly Gly Pro Val Arg Glu Pro Ala Leu Ser Val Ala Leu Trp
          20           25           30

Leu Ser Trp Gly Ala Ala Leu Gly Ala Val Ala Cys Ala Met Ala Leu
          35           40           45

Leu Thr Gln Gln Thr Glu Leu Gln Ser Leu Arg Arg Glu Val Ser Arg
          50           55           60

Leu Gln Gly Thr Gly Gly Pro Ser Gln Asn Gly Glu Gly Tyr Pro Trp
65           70           75           80

Gln Ser Leu Pro Glu Gln Ser Ser Asp Ala Leu Glu Ala Trp Glu Asn
          85           90           95

Gly Glu Arg Ser Arg Lys Arg Arg Ala Val Leu Thr Gln Lys Gln Lys
          100          105          110

Lys Gln His Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser Lys
          115          120          125

Asp Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg Arg
          130          135          140

Gly Arg Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp Ala
145          150          155          160

Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr Phe
          165          170          175

Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu Thr
          180          185          190

Leu Phe Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala Tyr
          195          200          205

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Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp Ile
 210 215 220
 Leu Ser Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser Pro
 225 230 235 240
 His Gly Thr Phe Leu Gly Leu
 245

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp
 1 5 10 15
 Arg Leu Ser Ala Glu Ile Asn Arg
 20

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Tyr Asn Xaa Cys Tyr Xaa Ala Gly Val Phe His Leu His Gln Gly Asp
 1 5 10 15
 Ile Leu Ser Val Ile Ile Pro Arg

20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Val	Pro	Ser	Glu	Gly	Leu	Tyr	Leu	Ile	Tyr	Ser	Gln	Val	Leu	Phe	Lys
1				5					10					15	

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ile	Gln	Asp	Ala	Gly	Val	Tyr	Leu	Leu	Tyr	Arg	Gln	Val	Leu	Phe	Gln
1				5					10					15	

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 233 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met	Ser	Thr	Glu	Ser	Met	Ile	Arg	Asp	Val	Glu	Leu	Ala	Glu	Glu	Ala	1	5	10	15
Leu	Pro	Lys	Lys	Thr	Gly	Gly	Pro	Gln	Gly	Ser	Arg	Arg	Cys	Leu	Phe	20	25	30	
Leu	Ser	Leu	Phe	Ser	Phe	Leu	Ile	Val	Ala	Gly	Ala	Thr	Thr	Leu	Phe	35	40	45	
Cys	Leu	Leu	His	Phe	Gly	Val	Ile	Gly	Pro	Gln	Arg	Glu	Glu	Phe	Pro	50	55	60	
Arg	Asp	Leu	Ser	Leu	Ile	Ser	Pro	Leu	Ala	Gln	Ala	Val	Arg	Ser	Ser	65	70	75	80
Ser	Arg	Thr	Pro	Ser	Asp	Lys	Pro	Val	Ala	His	Val	Val	Ala	Asn	Pro	85	90	95	
Gln	Ala	Glu	Gly	Gln	Leu	Gln	Trp	Leu	Asn	Arg	Arg	Ala	Asn	Ala	Leu	100	105	110	
Leu	Ala	Asn	Gly	Val	Glu	Leu	Arg	Asp	Asn	Gln	Leu	Val	Val	Pro	Ser	115	120	125	
Glu	Gly	Leu	Tyr	Leu	Ile	Tyr	Ser	Gln	Val	Leu	Phe	Lys	Gly	Gln	Gly	130	135	140	
Cys	Pro	Ser	Thr	His	Val	Leu	Leu	Thr	His	Thr	Ile	Ser	Arg	Ile	Ala	145	150	155	160
Val	Ser	Tyr	Gln	Thr	Lys	Val	Asn	Leu	Leu	Ser	Ala	Ile	Lys	Ser	Pro	165	170	175	
Cys	Gln	Arg	Glu	Thr	Pro	Glu	Gly	Ala	Glu	Ala	Lys	Pro	Trp	Tyr	Glu	180	185	190	
Pro	Ile	Tyr	Leu	Gly	Gly	Val	Phe	Gln	Leu	Glu	Lys	Gly	Asp	Arg	Leu	195	200	205	
Ser	Ala	Glu	Ile	Asn	Arg	Pro	Asp	Tyr	Leu	Asp	Phe	Ala	Glu	Ser	Gly	210	215	220	
Gln	Val	Tyr	Phe	Gly	Ile	Ile	Ala	Leu											

225

230

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 281 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Met Gln Gln Pro Phe Asn Tyr Pro Tyr Pro Gln Ile Tyr Trp Val Asp
1           5           10           15

Ser Ser Ala Ser Ser Pro Trp Ala Pro Pro Gly Thr Val Leu Pro Cys
          20           25           30

Pro Thr Ser Val Pro Arg Arg Pro Gly Gln Arg Arg Pro Pro Pro Pro
          35           40           45

Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro
          50           55           60

Pro Leu Pro Leu Pro Pro Leu Lys Lys Arg Gly Asn His Ser Thr Gly
65           70           75           80

Leu Cys Leu Leu Val Met Phe Phe Met Val Leu Val Ala Leu Val Gly
          85           90           95

Leu Gly Leu Gly Met Phe Gln Leu Phe His Leu Gln Lys Glu Leu Ala
          100          105          110

Glu Leu Arg Glu Ser Thr Ser Gln Met His Thr Ala Ser Ser Leu Glu
          115          120          125

Lys Gln Ile Gly His Pro Ser Pro Pro Pro Glu Lys Lys Glu Leu Arg
          130          135          140

Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser Met Pro Leu
          145          150          155          160

Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly Val Lys Tyr

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	165		170		175
Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr Phe Val Tyr					
	180		185		190
Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu Pro Leu Ser					
	195		200		205
His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp Leu Val Met					
	210		215		220
Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln Met Trp Ala					
	225		230		235
Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr Ser Ala Asp His					
	245		250		255
Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe Glu Glu Ser					
	260		265		270
Gln Thr Phe Phe Gly Leu Tyr Lys Leu					
	275		280		

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 281 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys			
1	5	10	15
Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala			
	20	25	30
Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys			
	35	40	45

Tyr	Ser	Lys	Ser	Gly	Ile	Ala	Cys	Phe	Leu	Lys	Glu	Asp	Asp	Ser	Tyr	50	55	60	
Trp	Asp	Pro	Asn	Asp	Glu	Glu	Ser	Met	Asn	Ser	Pro	Cys	Trp	Gln	Val	65	70	75	80
Lys	Trp	Gln	Leu	Arg	Gln	Leu	Val	Arg	Lys	Met	Ile	Leu	Arg	Thr	Ser	85	90	95	
Glu	Glu	Thr	Ile	Ser	Thr	Val	Gln	Glu	Lys	Gln	Gln	Asn	Ile	Ser	Pro	100	105	110	
Leu	Val	Arg	Glu	Arg	Gly	Pro	Gln	Arg	Val	Ala	Ala	His	Ile	Thr	Gly	115	120	125	
Thr	Arg	Gly	Arg	Ser	Asn	Thr	Leu	Ser	Ser	Pro	Asn	Ser	Lys	Asn	Glu	130	135	140	
Lys	Ala	Leu	Gly	Arg	Lys	Ile	Asn	Ser	Trp	Glu	Ser	Ser	Arg	Ser	Gly	145	150	155	160
His	Ser	Phe	Leu	Ser	Asn	Leu	His	Leu	Arg	Asn	Gly	Glu	Leu	Val	Ile	165	170	175	
His	Glu	Lys	Gly	Phe	Tyr	Tyr	Ile	Tyr	Ser	Gln	Thr	Tyr	Phe	Arg	Phe	180	185	190	
Gln	Glu	Glu	Ile	Lys	Glu	Asn	Thr	Lys	Asn	Asp	Lys	Gln	Met	Val	Gln	195	200	205	
Tyr	Ile	Tyr	Lys	Tyr	Thr	Ser	Tyr	Pro	Asp	Pro	Ile	Leu	Leu	Met	Lys	210	215	220	
Ser	Ala	Arg	Asn	Ser	Cys	Trp	Ser	Lys	Asp	Ala	Glu	Tyr	Gly	Leu	Tyr	225	230	235	240
Ser	Ile	Tyr	Gln	Gly	Gly	Ile	Phe	Glu	Leu	Lys	Glu	Asn	Asp	Arg	Ile	245	250	255	
Phe	Val	Ser	Val	Thr	Asn	Glu	His	Leu	Ile	Asp	Met	Asp	His	Glu	Ala	260	265	270	
Ser	Phe	Phe	Gly	Ala	Phe	Leu	Val	Gly								275	280		

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATATGGATCC CAGCTCATGC CAGCCTCA

28

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGTAAAGCTT GGAATTATGA CACTCAGAAT ATCCC

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(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CAGCTCATGC CAGCCTCA

18

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TATCCGTAAA ATCAAAGTCC CAG

23

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CATATGCACA GAGCTGCAGA GCCTCAGGAG

30

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TCACAGTTTC ACAAACCCCA GGAAG

25

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTAAAATCAA AGTCCCAGGA AGGT

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/18506

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/19 C07K14/52 C07K16/28 C07K16/24 C07K14/705
G01N33/68 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 33902 A (HUMAN GENOME SCIENCES, INC.) 18 September 1997 see page 46; figure 1; example 2	1,3-10, 12-18
A	see page 48 - page 49 see claim 30	19-23
A	WO 97 01633 A (IMMUNEX CORPORATION) 16 January 1997 see the whole document especially the examples	1-23

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

11 February 1999

Date of mailing of the international search report

24/02/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Le Cornec, N

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/US 98/18506

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WILEY S R ET AL: "IDENTIFICATION AND CHARACTERIZATION OF A NEW MEMBER OF THE TNF FAMILY THAT INDUCES APOPTOSIS" IMMUNITY, vol. 3, no. 6, December 1995, pages 673-682, XP000672297 cited in the application see page 678, right-hand column see page 680</p> <p style="text-align: center;">----</p>	1-23
E	<p>WO 99 00518 A (ABBOTT LABORATORIES) 7 January 1999 see sequence ID no.2 see claims</p> <p style="text-align: center;">----</p>	1-18
P,X	<p>HAHNE M. ET AL: "APRIL, a new ligand of the tumor necrosis factor family, Stimulates tumor cell growth" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 188, 1998, pages 1185-1190, XP002093077 see the whole document</p> <p style="text-align: center;">-----</p>	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/18506

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9733902 A	18-09-1997	AU 5366596 A	01-10-1997
WO 9701633 A	16-01-1997	AU 6340796 A	30-01-1997
		CA 2225378 A	16-01-1997
		EP 0835305 A	15-04-1998
		NO 976045 A	02-03-1998
		US 5763223 A	09-06-1998
WO 9900518 A	07-01-1999	NONE	